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Polimorfismi in geni della riparazione del DNA e suscettibilità al cancro colon-rettale

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Ad Alessio e ai miei genitori

CONTENTS

List of Abbreviation

AGT	O^6-alkylguanine (O^6-AG) DNA alkyltransferase
AP site	Apurinic/Apyrimidinic site
APC	Adenomatosis Polyposis Coli
ATM	Ataxiatelangiectasia Mutated
BER	Base Excision Repair
CCND1	Cyclin D1
CDKs	Cyclin-Dependent Kinases
CI	Confidence Interval
CRC	Colorectal Cancer
CS	Cockayne Sindrome
DDB	DNA Damage Binding protein
DNA	Deoxyribonucleic acid
DNA-PKcs	DNA dependent protein kinase catalytic subunit
DSB	Double Strand Break
FAP	Familial Adenomatous Polyposis
FEN1	Flap Endonuclease 1
GG-NER	Global Genome NER
HNPCC	Hereditary Non-Polyposis Colorectal Cancer
HRT	Hormone Replacement Therapy
HR	Homologous Recombination
MGMT	O^6-methylguanine methyltransferase
MMR	Mismatch Repair
NCR	National Cancer Registry
NER	Nucleotide Excision Repair
NHEJ	Non-Homologous End-Joining
NSAIDs	Non-Steroidal Anti-Inflammatory Drugs
O^6-AG	O^6-alkylguanine
O6-MeG	O^6-methylguanine
OR	Odds Ratio
PCNA	Proliferating Cell Nuclear Antigen
pRb	Retinoblastoma Protein
PTGS	Prostaglandin H Synthase
PPARg	Peroxisome Proliferator-Activated Receptor g
ROS	Reactive Oxygen Species
RNA	Ribonucleic acid
SNPs	Single-Nucleotide Polymorphisms
SSB	Single Strand Break
TC-NER	Transcription Coupled NER
TS	Thymidilate Synthase
TTD	Trichothiodystrophy
XME	Xenobiotic Metabolising Enzyme
XP	Xeroderma Pigmentosum
5′-dRP	5′ terminal deoxyribose phosphate

ABSTRACT

Polymorphisms in DNA repair genes and susceptibility to colorectal cancer

Colorectal cancer represents a complex disease where susceptibility may be influenced by genetic polymorphisms in the DNA repair system and in the cell cycle control pathway. In the present study we investigated the role of nine single nucleotide polymorphisms in eight DNA repair genes on the risk of colorectal cancer in a hospital-based case-control population (532 cases and 532 sex- and age-matched controls) from the Czech Republic. A similar investigation on the same cohort was done for three single nucleotide polymorphisms in cell cycle control genes (*TP53* PIN3 and Arg72Pro, *CCND1* G/A870). Data analysis showed that the variant homozygote for the Asn148Glu polymorphism in the *APE1* gene was associated with a moderately increased risk of colorectal cancer, but the association was more pronounced for colon cancer (OR: 1.50, 95% CI 1.01-2.22; p=0.05). The data stratification according to age showed increased risk of colorectal cancer in the age group 64-86 years in individuals heterozygous (OR: 1.79, 95% CI 1.04-3.07; p=0.04) and homozygous (OR: 2.57, 95% CI 1.30-5.06; p=0.007) for the variant allele of the *APE1* Asn148Glu polymorphism. Smokers homozygous for the variant allele of the *hOGG1* Ser326Cys polymorphism showed increased risk of colorectal cancer compared to smokers carrying *hOGG1* 326SerSer genotype (OR: 4.17, 95% CI 1.17-15.54; p=0.03). The analysis of binary genotype combinations showed increased colorectal cancer risk in individuals simultaneously homozygous for the variant alleles of *APE1* Asn148Glu and *hOGG1* Ser326Cys in comparison to individuals carrying simultaneously the combination of homozygous genotypes for the wildtype alleles of the same genes (OR: 6.37, 95% CI 1.40-29.02; p=0.02). Oldest patients exhibited also significantly increased risk of colon and rectal cancer due to genotype combinations of *APE1* Asn148Glu and *XPG* Asn114His. No significant association emerged from cell cycle gene polymorphisms analyzed in this study (*TP53* PIN3 and Arg72Pro, *CCND1* G/A870). The analysis of binary genotype combinations showed increased colon cancer risk in individuals simultaneously homozygous for the variant alleles of the *TP53* polymorphisms (PIN3

and Arg72Pro). Considering the subtle effect of the DNA repair polymorphisms on the risk of colorectal cancer, exploration of gene-gene and gene-environmental interactions requires large sample size for sufficient statistical power.
Keywords: Colorectal cancer, individual susceptibility, DNA repair, single-nucleotide polymorphisms, case-control study

RIASSUNTO

Il cancro colon-rettale rappresenta un tipo di malattia molto complessa la cui suscettibilità può essere influenzata dai polimorfismi a geni del sistema di riparazione del DNA o a geni implicati nel controllo del ciclo cellulare. Nel presente studio è stato analizzato il possibile ruolo di nove polimorfismi a singolo nucleotide in otto geni facenti parte del sistema di riparazione del DNA nell'incremento del rischio di cancro colon-rettale in una popolazione di casi e controlli proveniente dalla Repubblica Ceca (532 casi e 532 controlli appaiati per sesso ed età). Una simile analisi sulla stessa popolazione è stata eseguita per quanto riguarda l'effetto di tre polimorfismi a singolo nucleotide di due geni implicati nel controllo del ciclo cellulare (*TP53* PIN3 and Arg72Pro, *CCND1* G/A870) nella suscettibilità al cancro colon-rettale.

Dall'analisi dei risultati è emerso che il genotipo omozigote variante per il polimorfismo Asn148Glu al gene *APE1* è risultato associato con un moderato incremento del rischio per il tumore al colon-retto, l'associazione risultava più pronunciata per il rischio del solo tumore al colon (OR: 1.50, 95% CI 1.01-2.22; p=0.05). Dall'analisi stratificata in accordo con l'età alla diagnosi è emerso un incremento statisticamente significativo nel rischio di manifestazione di un cancro colon-rettale nel gruppo di individui di età compresa tra 64 e 86 anni e portanti il genotipo eterozigote (OR: 1.79, 95% CI 1.04-3.07; p=0.04) ed omozigote (OR: 2.57, 95% CI 1.30-5.06; p=0.007) per l'allele variante del polimorfismo di *APE1* Asn148Glu. Fumatori che presentavano il genotipo omozigote variante per il polimorfismo Ser326Cys del gene *hOGG1* presentavano un incremento significativo nel rischio per questo tipo di tumore se confrontati con la popolazione di fumatori di controllo (OR: 4.17, 95% CI 1.17-15.54; p=0.03).

Dall'analisi delle combinazioni binarie di polimorfismi a geni facenti parte dello stesso pathway di riparazione del DNA è emerso un incremento significativo nel rischio di cancro colon-rettale per gli individui portatori simultaneamente dei genotipi omozigoti per gli alleli varianti dei polimorfismi *APE1* Asn148Glu e *hOGG1* Ser326Cys (OR: 6.37, 95% CI 1.40-29.02; p=0.02). La sottopopolazione di individui più anziani (64-86 anni) presentava, inoltre, un incremento nel rischio di cancro al colon e al retto quando

presenti particolari combinazioni di genotipi per i polimorfismi *APE1* Asn148Glu e *XPG* Asn114His. Nessuno dei polimorfismi ai geni del ciclo cellulare analizzati (*TP53* PIN3 and Arg72Pro, *CCND1* G/A870) è stato associato con un incremento significativo del rischio di tumore colon-retto. Dall'analisi delle combinazioni binarie è stato osservato, tuttavia, un incremento significativo nel rischio di cancro al colon per gli individui portatori simultaneamente dei genotipi omozigoti per gli alleli varianti dei polimorfismi al gene *TP53* (PIN3 and Arg72Pro). I risultati emersi dal presente studio suggeriscono che ci possa essere un sottile effetto modulatore dei polimorfismi dei geni della riparazione del DNA nella suscettibilità al cancro colon-rettale, soprattutto nel caso di particolari combinazioni di genotipi "sfavorevoli". L'analisi di tali interazioni gene-gene e di eventuali interazioni dei geni con l'ambiente richiedono, però, popolazioni di studio più ampie per avere un sufficiente potere statistico.

1. Introduction

1.1 Colorectal cancer: importance and causes

Colorectal carcinoma (CRC) is one of the most frequent causes of cancer death in industrialized countries, with a yearly incidence of about 50 new cases for every 100.000 people in the population, and an individual lifetime risk approaching 5% (Boyle and Ferlay 2005, Jemal et al., 2006). The well-defined sequence of events in this tumour development, from aberrant crypt proliferation or hyperplasia to benign adenomas, then to carcinoma *in situ* and finally to metastatic carcinoma, attests to the step like progression of this cancer, which often occurs over many years (de la Chapelle, 2004). These distinctive morphological stages can be visualized by colonoscopy, enabling the removal of pre-malignant lesions and of early malignant tumors.

CRC is traditionally divided into sporadic and familial (hereditary) forms, the proportion of familial CRC accounting for 20–25% of the total number of cases. The majority of CRC cases is sporadic or shows a pattern of familial aggregation, not easily fitting into models of Mendelian inheritance (de la Chapelle, 2004). Genetics plays a key role in the susceptibility, initiation and progression of CRC. Genetic syndromes predisposing to CRC include the polyposis syndromes (familial adenomatous polyposis (FAP), Peutz-Jeghers syndrome, and Juvenile polyposis) and hereditary non-polyposis colon cancer (HNPCC). However, these syndromes account for about 3% of all cases (Aaltonen et al., 1998, Samowitz et al., 2001) and are not responsible for the 2-fold increased risk in first-degree relatives of sporadic CRC patients. This increased risk in relatives suggests a possible mild genetic predisposition, such as, for instance, an involvement of low-penetrance genes or gene combinations.

CRC is a complex disease, whose development is determined by different combinations of genetic and environmental causes. These include on one hand dietary and lifestyle habits and on the other hand genetic factors (Potter, 1999). Rare and highly penetrant mutations in cancer genes may act with environmental influence. As the majority of CRC cases are sporadic, environmental factors are

thought to have an important role in the development of this disease. Increasingly large epidemiological studies have contributed to highlight the role of diet and lifestyle in humans over the last 40 years: they have established a positive correlation between CRC risk and the intake of fat, red meat and alcohol, as well as smoking (Giovannucci, 2001, Norat and Riboli, 2001). On the contrary, an inverse correlation has been observed between CRC risk and intake of vegetables and fibres (Terry et al., 2001, Young et al., 2005). Other non-dietary environmental life-style factors with protective effects include: higher levels of physical activity, hormone replacement therapy (HRT) in postmenopausal females, and the regular use of non-steroidal anti-inflammatory drugs (NSAIDs), such as aspirin (Greenberg et al, 1993, Giacosa et al., 1999). Many associations are still controversial, and in most cases little is known about the mechanisms that contribute to enhance/diminish cancer risk. The difficulties in understanding main risk factors in CRC onset can be due to the fact that there are not single events predisposing to cancer development, but a sort of tight interaction between genetic and environmental factors with different degrees of involvement of either. Various situations may occur: tumorigenesis can require a close interaction between genetic risk factors and specific exposure to initiate the procedure or, genetic and environmental factors can complement and/or enhance each other, or, in an opposite situation, one of the two components can be protective in respect to the other. It is believed that a chronic disease, such as sporadic CRC, more likely involves multiple genes with moderate effects (low penetrance type) and progress materializes due to aggressive gene-environment interactions (Ahmed, 2006). Thus, it is reasonable to expect also the presence of several low-penetrance genes, which may influence the effects of both genetic and environmental factors contributing to CRC susceptibility. These genes, although none of them has been clearly identified, may account for either cases of hereditary CRC, or mainly for cases of CRC that are classified as ‘sporadic’. Therefore, the distinction between ‘sporadic’ and ‘familial’ cases and between ‘genetic’ and ‘environmental’ predisposing factors has become blurred, and might be better thought of as a continuum of risks contributing to CRC development (de la Chapelle, 2004).

Primary and secondary prevention of CRC are very important: this is proven by the fact that although its prevalence has been steadily increasing over the last century, mortality rates have declined, as a consequence of both improved treatment and

efficient screening and surveillance (Jemal et al., 2006). Occult fecal blood testing is a widely used and relatively simple option, but its sensitivity and specificity are still far from satisfactory. Endoscopy is certainly a more efficient tool, but its invasive nature and associated risks hamper its application on a population-wide scale. Interventions associated with changes in dietary and lifestyle habits (primarily smoking and alcohol consumption) and environmental exposure are, of course, strategies that would also reduce the risk of CRC, but again, it is extremely difficult to operate on population-wide scale (Heavey et al., 2004). The ultimate goal of an extensive primary prevention would be a stratification of the population into CRC risk categories, which could allow targeted prevention, with the adoption of measures tailored according to individual risk levels. In order to achieve this goal, accurate knowledge on the etiology and pathogenesis of the disease is needed, as well as the relationships between an individuals` genetic background and the relevance of environmental factors (Baglioni and Genuardi, 2004).

Colorectal cancer incidence in the Czech Republic: a negative record

High incidence of CRC is concentrated in developed countries and there is a high mortality in the countries of Central and Eastern Europe. The underlying cause may be mainly insufficient prevention, unhealthy nutrition and bad life style, which can lead to higher frequency of the disease. CRC represents a serious health problem in particular in the Czech Republic, as the incidence for colon cancer ranks the third highest worldwide and the incidence of rectal cancer is the highest (Boyle and Langman, 2000, Janout and Kollarova, 2001, Parkin et al, 2005) (**Figure 1 and 2**).

Malignant neoplasms have been registered in the Czech Republic since the late 1950s, with the establishment of the National Cancer Registry of the Czech Republic (NCR) in 1976 (Cancer Incidence 2002 in the Czech Republic). Compared to other European countries, the Czech Republic presents a rather long and historical recording of cancer incidence. Colorectal malignant neoplasms are the second most frequent kind of cancer recorded in this country (last updated calculation 31/12/2002). In 2002 there were 8022 reported cases, accounting for 12.3 % of all reported malignant neoplasms and carcinoma *in situ*. It affects both men and women, with somewhat higher incidence among the former (58.7 %). The gravity of this illness is confirmed by the fact that it ranks second both in incidence and in mortality (15.6 % of all deaths from cancer were connected with colorectal cancer).

Mortality of men from CRC is the highest worldwide. Mortality among women is only exceeded by Hungarian women. (Cancer Incidence 2002 in the Czech Republic).

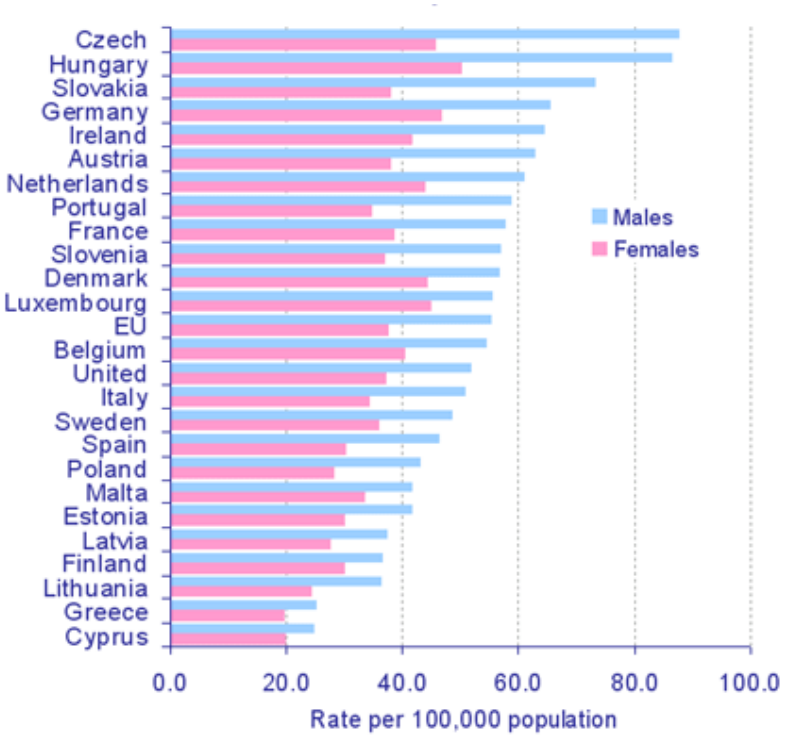

Figure 1 Age standardized (European) incidence rates, bowel cancer in EU countries, by sex, 2002 estimates (*Modified from IARC, GLOBOCAN 2002. Cancer Incidence, Mortality and Prevalence Worldwide (2002 estimates) 2005*)

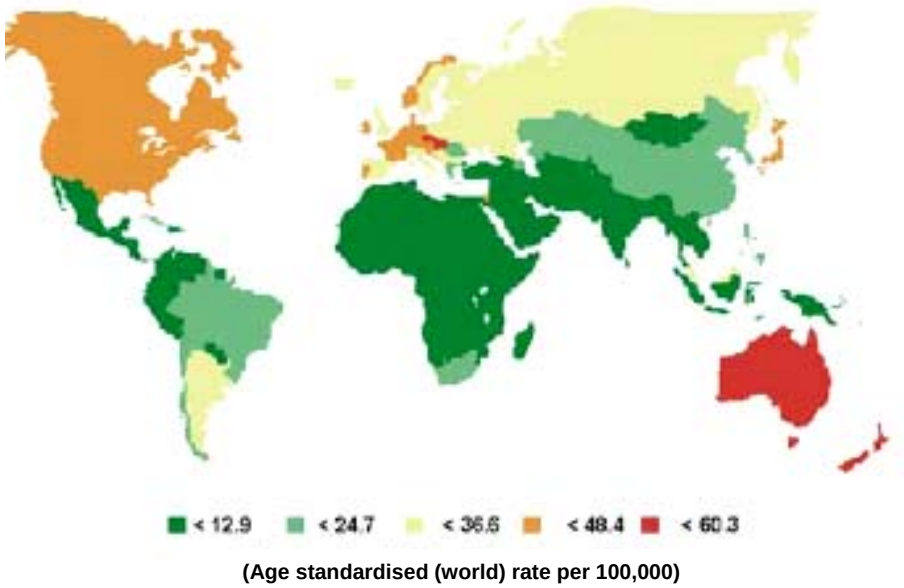

Figure 2 Worldwide incidence of colorectal in males (*Modified from Parkin et al, 2005*)

1.2 Importance of low-penetrance genes in CRC

The identification of polymorphisms (the occurrence in the same population of multiple discrete allelic states of which at least two have high frequency, conventionally of 1% or more) in genes associated with carcinogen metabolism has stimulated several years ago the development of a hypotheses to explain the high degree of individual variability in cancer susceptibility that has been observed by epidemiologists (Vineis, 2004). The successful sequencing of the human genome has provided the identification of a large number of low-penetrance alleles and simultaneously molecular epidemiology has acquired the technological devices to design large-scale case-control association studies. However, many results from epidemiologic studies have been inconsistent, particularly because of 1) low statistical power for detecting a moderate effect, mainly due to small size of analyzed cohorts, 2) false-positive results, 3) heterogeneity across study populations, like ethnic differences in genetic background, 4) failure to consider effect modifiers such as environmental exposures, and 5) publication bias versus negative results (Wacholder et al., 2004). In addition, it is becoming clear that a major need in association studies is to identify biologically plausible functional reflections of many polymorphisms, as well as gene-gene and gene-environment interactions (Rebbeck et al., 2004). Interaction between dietary and other environmental factors and single-nucleotide polymorphisms (SNPs) in specific genes have been proposed to explain much of the inter-individual variations in cancer, and provide a mechanistic explanation for the lack of evidence of effect of some promising dietary factors in chemoprevention studies (**Figure 3**).

The number of candidate genes in the case of CRC, i.e. genes that may influence the development of this type cancer, is extremely wide and many of them possess known high-frequency low-penetrance alleles (de La Chapelle, 2004). Genes implicated in metabolic pathways, in methylation, or in DNA repair, as well as oncogenes, tumor suppressor genes, genes modifying the colonic microenvironment, and genes involved in the immune response have been so far addressed in this respect as a candidate gene for CRC susceptibility (de Jong, 2002). Low-penetrance variants in high-penetrance genes (e.g., *APC*, *MLH1*, or *MSH2*) might also be important in sporadic and familial CRC. The *APC* Ile1307Lys polymorphism, for instance, represents the strongest case for the existence of CRC susceptibility alleles

conferring moderate risk increase. Although the functional properties of the APC protein are not modified by the presence of a lysine instead of an isoleucine, the underlying DNA sequence change, i.e. a substitution of a thymine for an adenine, determines the appearance of a short hypermutable poly-A repeat (Laken et al., 1997). This increases the likelihood that a first somatic hit, represented by *APC* sequence frameshifts, will occur in somatic cells, thus determining a higher CRC risk. Several studies have reported that the high frequency of *APC* 1307Lys variant among individuals of Ashkenazi descent corresponds to an approximately two-fold lifetime risk of CRC compared to the general population (Gryfe et al., 1999, Rozen et al., 2002).

The following part provides a brief overview of the main pathways in which genetic polymorphisms have been investigated in association with CRC risk.

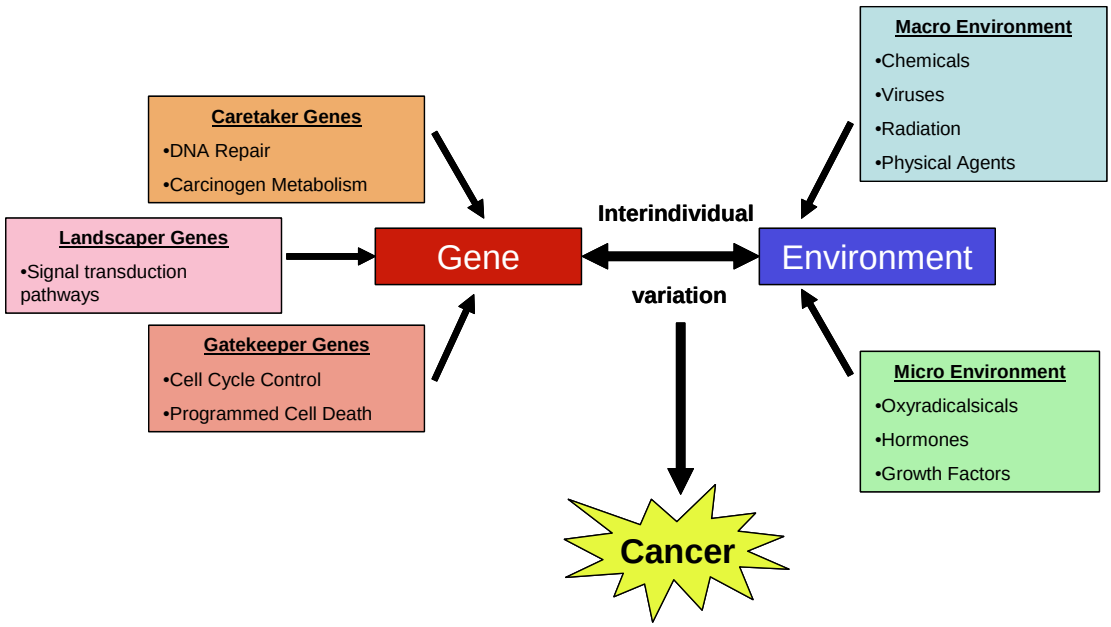

Figure 3 The possible interactions of genes with external/internal environmental factors modulate individual genetic susceptibility in sporadic CRC risk *(modified from Ahmed, 2006).*

- ***Metabolism***

Xenobiotic in the body are activated or inactivated by metabolic enzymes (XMEs). Most XMEs gene have several genetic polymorphisms, which can affect their activities, and modify metabolic pathways. As proposed by Vainio et al (2002), the general host metabolic status can be a crucial factor that enhances or reduces cancer

progression. The results of a meta-analysis on association studies about XME polymorphisms and risk of CRC have showed that *GSTT1* deletion is a risk factor for this kind of cancer together with *NAT2*-rapid acetylator phenotype and genotype and *NAT2*-rapid acetylator phenotype (Chen et al. 2005),. However, the authors concluded that since CRC is also associated with several environmental and dietary risk factors that should be always carefully considered in the relationship with genetic polymorphisms of metabolic enzymes.

- ***Methylation***

Imbalanced DNA methylation, characterized by genomic hypomethylation (Goelz et al., 1985) and methylation of usually unmethylated CpG sites (Issa et al., 1994), is observed consistently in CRC. The 5,10 MTHFR enzyme plays the most important role in DNA methylation and synthesis (Powers, 2005). The most common polymorphisms in the *MTHFR* gene, Cys677Thr and Ala1298Cys, have been frequently analyzed in association with CRC and adenoma risk. Recently, based on the evaluation of 16 studies, a decreased risk has been observed for carriers of the 677ThrThr genotype in the majority of the cases, independently from the low/high folate status. On the contrary inconclusive results emerged for the Ala1298Cys polymorphism, probably due to the limited number of data available so far (Kono and Chen, 2005).

- ***Immune response genes***

Chronic inflammation has a relevant impact on crucial cellular processes such as proliferation, adhesion, apoptosis, angiogenesis, and transformation processes and has been implicated as one of the most important factors in the development of CRC. At the cellular level, the colon epithelium is exposed to a variety of toxic and pathogenic agents. Alterations of intestinal microflora may result in a change in immune response, including the induction of inflammation (Macdonald and Monteleone, 2005). The release of proinflammatory cytokines by infiltrating lymphocytes usually accompanies these processes, leading to the generation of reactive oxygen species and other genotoxic compounds in the epithelial environment. Several studies indicate that local immunoregulation and associated cytokines have a putative role in the development of cancer, especially for gastrointestinal tumors. Genetic polymorphisms of the related cytokine encoding genes directly influence inter-individual variation in the magnitude of response, and

this may clearly contribute to the individual ultimate clinical outcome (MacArthur et al., 2004). A striking example is provided by *Helicobacter pylori*–induced gastritis, in which particular alleles for these proinflammatory genes have been associated with an increased risk of gastric cancer (El-Omar et al., 2001). Several studies have investigated the possible associations between cytokines and CRC risk. Common variants of proinflammatory cytokines IL6, IL8, and IL10 have been associated with CRC risk (Landi et al., 2003, MacArthur et al. 2004, Viet et al., 2005).

Interesting associations have also been reported for polymorphisms of other genes involved in inflammatory processes, like the prostaglandin H synthase (PTGS, also known as cyclooxygenase, catalyzing the conversion of arachidonic acid to prostaglandin precursors, and mediating a wide range of cell functions) and the peroxisome proliferator-activated receptor g (PPARg, a nuclear receptor that functions as a transcriptional regulator of metabolism). Allelic variants of PTGS2 have been investigated with colorectal carcinomas and adenomas with conflicting outcomes in several studies (Lin et al., 2002, Ali et al., 2005), while a variant within the PTGS2 promoter region has been recently associated with colorectal adenoma among non-NSAID users (Ulrich et al., 2005). Also a common PPARg SNP in exon 12, which leads to a nonsynonymous amino acid substitution, has been associated with CRC and colorectal adenoma risk (Landi et al., 2003, Gong et al., 2005).

- ***Oncogenes and Tumor Suppressor genes***

The proto-oncogene *HRAS1* encodes a protein involved in mitogenic signal transduction and differentiation, and the *HRAS1* gene is highly polymorphic in the human population (Heim and Mitelman, 1987). The few available studies on the *HRAS1* polymorphisms are heterogeneous; the number of different *HRAS1* alleles range from 5 up to 20 according to different authors, and this situation makes it difficult to compare data obtained in different laboratories. However, in most studies there are four common alleles, whereas the rest of the variant alleles are rare. The variant *HRAS1* alleles have been associated with a moderately increased CRC risk (Klingel et al., 1991, Gosse-Brun et al., 1998).

Gene polymorphisms were also shown to affect: (a) growth control at the cellular level (cell proliferation, differentiation and death); (b) factors involved in tumor invasion and metastasis (extracellular matrix remodeling, angiogenesis and cell

adhesion); (c) action of hormones and vitamins on growing tumors; and (d) outcome of cancer therapy (pharmacogenetics) (Ahmed, 2006).

Other processes in which genetic polymorphisms have been investigated in association with CRC risk are DNA repair and Cell-cycle control. The following section provides an overview on these two important pathways.

1.3 DNA repair

The genome is continuously attacked by endogenous and exogenous mutagens. Unrepaired damage can ultimately result in apoptosis or may lead to unregulated cell growth and cancer. When the DNA damage is recognized by cell machinery, several responses may occur to prevent replication in the presence of genetic errors: checkpoints can be activated to arrest the cell cycle, transcription can be up-regulated to compensate for the damage, or the cell can undergo apoptosis. Alternatively, the damage can be repaired at the DNA level enabling the cell to replicate. The individual DNA repair capacity is a complex system of defenses designed to protect the integrity of the genome. DNA repair is thus of primary importance in the general and specialized functions of cells, as well as in the prevention of carcinogenesis (Hoeijmakers, 2001, Kaina, 2003).

DNA repair is commonly divided into five major pathways (direct damage reversal, base excision repair (BER), nucleotide excision repair (NER), mismatch repair (MMR), and double strand break repair (DSB repair)), each dealing with specific types of lesions (Gillet and Scharer 2006).

Nucleotide Excision Repair (NER)

NER is a particularly intriguing repair pathway due to its extraordinarily wide substrate specificity. It has the ability to recognize and repair a large number of structurally unrelated lesions, such as DNA damage induced by UV radiation from sunlight and numerous bulky DNA adducts induced by environmental xenobiotics or by cytotoxic drugs used in chemotherapy. Defects in NER are related to various pathologies: a) Xeroderma Pigmentosum (XP), a recessive genetic disease that induces in XP patients an extreme sensitivity to sunlight and a more than 1000-fold increased risk to develop skin cancer; b) Cockayne syndrome (CS) is characterized by UV sensitivity but also by additional symptoms such as short stature, severe neurological abnormalities caused by dysmyelination, bird-like faces, tooth decay, and cataracts. CS patients have a mean life expectancy of 12.5 years but in contrast to XP do not show a clear predisposition to skin cancer; c) Trichothiodystrophy (TTD), (literally: “sulfur-deficient brittle hair”), in addition to the symptoms shared with CS patients, TTD patients show characteristic sulfur-deficient, brittle hair and

scaling of the skin. This genetic disorder is now known to correlate with mutations in genes involved in NER (such as *XPB*, *XPD*, and *TTDA* genes) (Gillet and Scharer 2006)

NER operates through a "cut-and-patch" mechanism in 4 stages:

1. DNA damage recognition by a protein complex including XPC;
2. Unwinding of DNA helix by TFIIH complex which includes XPD;
3. Excision and removement of a short stretch of DNA (24- to 32-nucleotides long) containing the damaged base by several molecules and complexes that includes XPF-ERCC1;
4. The original genetic sequence is then restored using the non-damaged strand of the DNA double helix as a template for repair synthesis.

In eukaryotic cells the process of NER requires more than 30 proteins (Van Hoffen et al., 2003).

Two distinct subpathways have been discerned: global genome NER (GG-NER), which can detect and remove lesions throughout the genome, and transcription coupled NER (TC-NER), which ensures faster repair of many lesions when located on the transcribed strand of actively transcribed genes (**Figure 4**).

- **Global Genome NER (GG-NER)**

GG-NER is considered to be transcription-independent, removing lesions from non-transcribed regions of genome in addition to non-transcribed strands of transcribed regions. At least two proteins have been identified and implicated in the lesion recognition step of NER: the XPC gene product in complex with HR23B and the DNA damage binding protein (DDB) complex consisting of a heterodimer of the XPE, protein p48 and p127. The XPC-HR23B complex has strong specific affinity for damaged DNA, directly binding to the lesion. It is essential for the formation of the incision complex. DDB is required for repair of moderately helix distorting DNA lesions. The damage recognition by the XPC-HR23B complex is greatly facilitated by the DDB complex (Van Hoffen et al. 2003; Gillet and Scharer 2006).

- **Transcription Coupled NER (TC-NER)**

The preferential repair of UV-induced damage in transcribed strands of active genes is known as TC-NER. Impairment of the ability for TC-NER results in the onset of an autosomal recessive CS disease, characterized by hypersensitivity to UV light. TC-NER depends on active RNA polymeraseI and II driven transcription and is specifically targeted to DNA lesions in the transcribed strand of an active gene.

DNA lesions that induce poor helix distortion can block the movement of elongation by RNA Pol II. This can lead RNA Pol II to stalling and subsequent triggering of repair mechanisms by TC-NER resulting in an accelerated repair of DNA lesion in the transcribed strand compared to repair of non-expressed DNA. CSA and CSB gene products recognize the damage in that case. *CSB* gene encodes for a 168 kDa protein with particular importance, since its mutations may abolish completely TC-NER systém, causing the block of RNA polymerase (Van Hoffen et al. 2003; Gillet and Scharer 2006).

The sequential steps following the initial recognition of DNA lesions by GG-NER and TC-NER are similar.

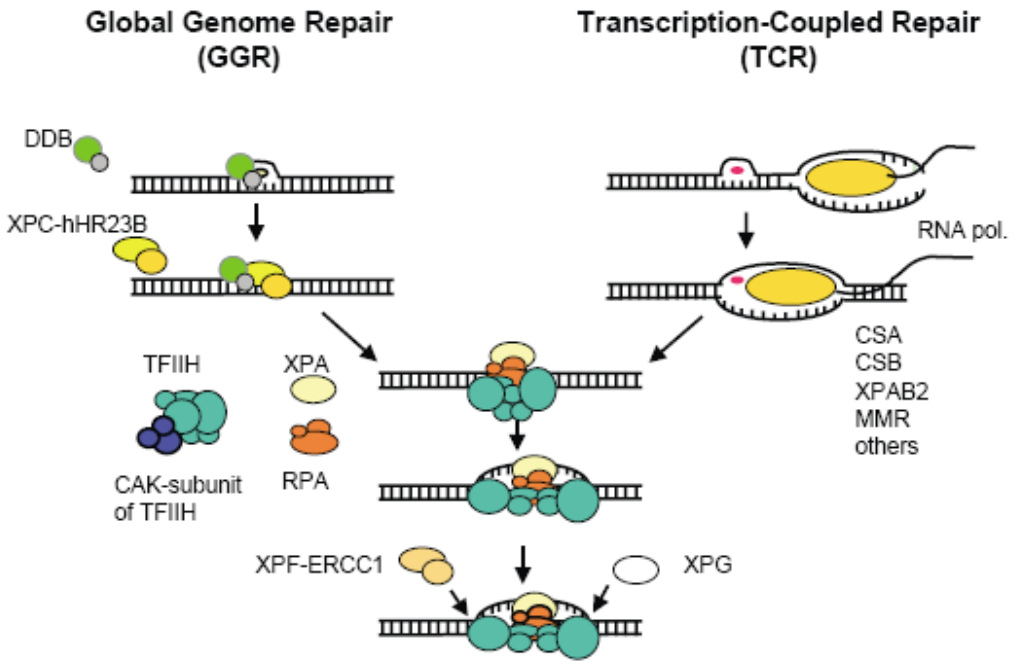

Figure 4 NER in mammalian cells. The damage recognition step is different for GGR and TCR. Once the damage is recognized, the processing of the damage requires the same steps and proteins in both subpathways (*Modified from Van hoffen et al, 2003*).

XPA, RPA, XPC-HR23B, and DDB were found to exhibit an affinity for damaged DNA and were thus proposed to be involved in the damage recognition step of NER (**Figure 4**). XPB and XPD were identified as two ATP dependent helicases (unwinding 3′-5′and 5′-3′duplex DNA, respectively), subunits of the transcription

factor IIH (TFIIH) complex involved in basal transcription. This suggested a novel function for the whole TFIIH complex during the NER process, in generating an open DNA structure around the lesion. ERCC1- XPF and XPG were found to be structure-specific endonucleases that incised DNA at single-stranded/double-stranded junctions with a specific polarity. ERCC1-XPF and XPG were thus proposed as the two nucleases performing the dual incision on the damaged strand, respectively, 5′- and 3′- to the lesion. PCNA (proliferating cell nuclear antigen) has also been shown to be required for NER in vitro, i.e. for the DNA resynthesis step, suggesting that DNA polymerase δ or ε is involved in NER, most probably in the last steps before completing repair process (Gillet and Scharer, 2006).

Base Excision Repair (BER)

BER is fundamentally important in handling diverse lesions produced as a result of the intrinsic instability of DNA or by various endogenous and exogenous reactive species. The initial step of BER involves enzymatic activities that process the N-glycosylic bonds linking the target bases and their deoxyribose sugars. DNA glycosylases acting on other diverse lesions (alkylated, oxidized, or photodamaged bases, as well as some mispaired bases) have been characterized for their biochemical properties and biological roles in BER. Mammalian cells contain at least 10 distinct glycosylase activities. For example, the product of the *OGG1* gene catalyzes the excision of a modified base, 8-oxoguanine, from DNA that has been damaged by exposure to reactive oxygen species; reduced ability to excise 8-oxoguanine may lead to an accumulation of oxidation-induced mutations (Goode et al, 2002; Ide and Kotera, 2004).

The initial product of a DNA glycosylase is an abasic, apurinic⁄apyrimidinic (AP), site in DNA, which is the central intermediate during BER (Sung and Demple, 2006).

A simplified version of BER for AP sites can be described as follows: 1) enzymatic incision of the AP site; 2) excision of the cleaved AP site at the single-strand break; 3) repair DNA synthesis; 4) ligation of the nick in DNA. In mammalian cells, the major AP endonuclease, APE1, hydrolyzes the 5′ phosphodiester bond of the AP site to generate a DNA repair intermediate that contains a single strand break with 3′-hydroxyl and 5′-deoxyribose- 5-phosphate (5′-dRP) termini. Further repair is achieved through at least two distinct BER subpathways that involve different

subsets of enzymes, and which result in the replacement of one nucleotide (short-patch BER), or two or more nucleotides (long-patch BER) (**Figure 5**).

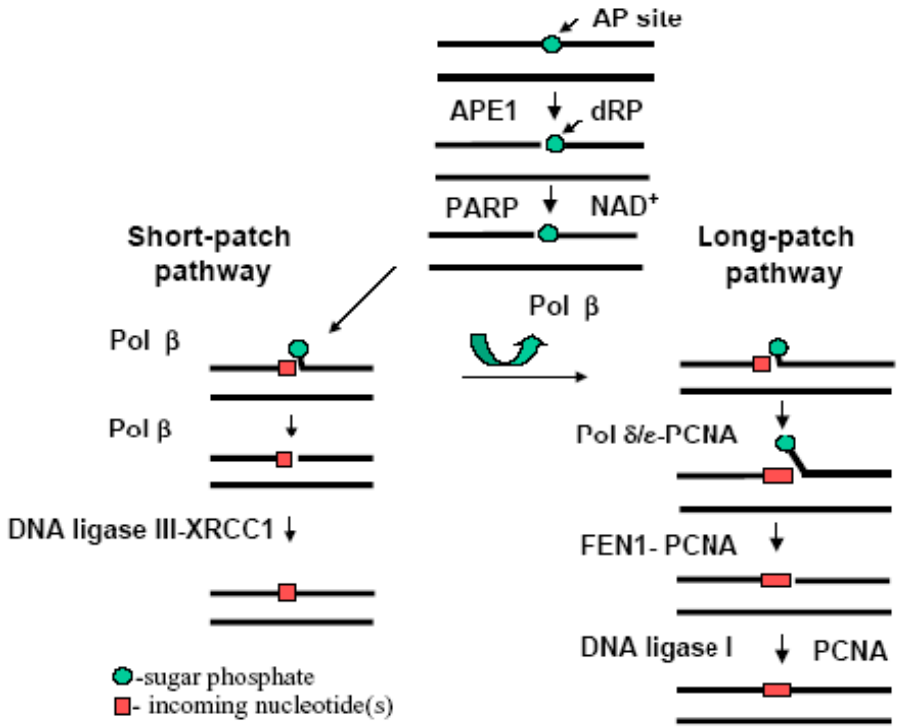

Figure 5 BER in mammalian cells. BER acts on small lesions and involves release of the damaged base and removal of up to a few neighboring nucleotides. Two distinct BER subpathways are described *(Modified from Sung and Demple, 2006)*.

The resulting 3′-OH terminus is extended by Pol β and at the same time 5′-dRP terminal is removed by AP lyase activity associated with Pol β , and finally the nick is sealed by DNA ligase III/XRCC1. The net reaction in this BER process is the replacement of a single nucleotide unit, and is called short-patch BER. In the other subpathway of BER, long-patch BER, Pol δ or Pol ε synthesizes several nucleotides by displacing the downstream strand containing 5′-dRP. The resulting flap structure bearing 5′-dRP is incised by flap endonuclease (FEN1), and the nick is sealed by DNA ligase I. PCNA interacts with Pol δ/ε, FEN1, and DNA ligase I throughout the process, supporting their functions. The long patch BER pathway appears to have a crucial role in processing oxidized or reduced AP sites that are resistant to the AP lyase activity of Pol β (Ide and Kotera, 2004; Sung and Demple, 2006).

Double-strand breaks repair (DSB Repair)

The DSB is perhaps the most dangerous of the various types of DNA damage that can occur within the mammalian cell. DSBs are induced by ionizing radiation (X-rays or γ-rays) as well as by radiomimetic drugs used for chemotherapy. Moreover, the by products of cellular metabolism (reactive oxygen species or ROS) can also induce these breaks. DSB are also generated when a replication fork passes through a template with a nick. Ultimate result is the generation of free oxygen radicals which can break the phosphodiester bonds in the DNA backbone. Two such breaks on opposite strands of DNA, located closely to each other, result in a DSB.

DNA DSB, as opposed to single-strand nicks or base modifications, can easily lead to gross chromosomal aberrations if not rejoined quickly. Even if repaired quickly, the repair process may be error-prone, and may eventually be detrimental to the organism. Mammalian cells, therefore, have mechanisms for quickly transmitting the damage signal to the cell cycle arrest or apoptotic machineries and mechanisms for DNA repair. Cell cycle arrest is necessary for providing the cell enough time for repair and, in some cases, it may be more prudent for the cell to undergo apoptosis when faced with excessive or unrepairable DNA damage. Both above processes represent effective barriers to carcinogenesis. The other important barrier to genomic instability and carcinogenesis is DSB repair (Burma et al, 2006)

Repair of DSB is intrinsically more difficult than other types of DNA damage because no undamaged template is available (Khanna and Jackson, 2001). At least two pathways of DSB are arbitrarily recognized: the homologous recombination (HR) pathway and the non-homologous end-joining repair (NHEJ) pathway. HR is operative only in the S/G2 phases of the cell cycle when a sister chromatid is available. NHEJ, which simply pieces together the broken DNA ends, can function in all phases of the cell cycle and is the predominant repair pathway in mammalian cells.

- ***Homologous recombination (HR) pathway***

HR is generally an error-free pathway of homology-directed repair. A DSB is accurately repaired by using the undamaged sister chromatid as a template for the repair of the broken sister chromatid (**Figure 6**). Homologous recombination in eukaryotes is carried out by the RAD52 epistasis group of proteins. In human cells, the proteins in this group include the MRN (RAD50/MRE11/NBS1) complex, RAD51, the RAD51 paralogs (RAD51B, RAD51C, RAD51D, XRCC2, XRCC3),

RAD54 and RAD54B. The products of the breast cancer susceptibility genes, BRCA1 and BRCA2, are also involved in the modulation of the homologous recombination (Agarwal et al, 2006).

When a DSB is detected, the initial damage response is mediated through the MRN complex and Ataxia telangiectasia mutated protein (ATM). The resection of DNA ends is required to generate 3´ single-stranded DNA tails, which are the substrate for homologous recombination, because they are utilized for the nucleation of recombination factors on the DNA. RAD51 is an important protein at the core of homologous recombination.With the help of accessory factors, RAD51 polymerizes on the 3´ tails to create a nucleoprotein filament. After a homology search, the nucleoprotein filament invades the target duplex at the site of homology to create a critical intermediate, the D-loop. This joint molecule between the broken sister chromatid and the intact sister chromatid is used as a template for DNA polymerases. In this way the sequence information that was lost in the initial processing of the DSB end is restored. The reaction is terminated with the ligation of DNA strands and the separation of the joint molecules to yield two intact DNA copies (Agarwal et al, 2006).

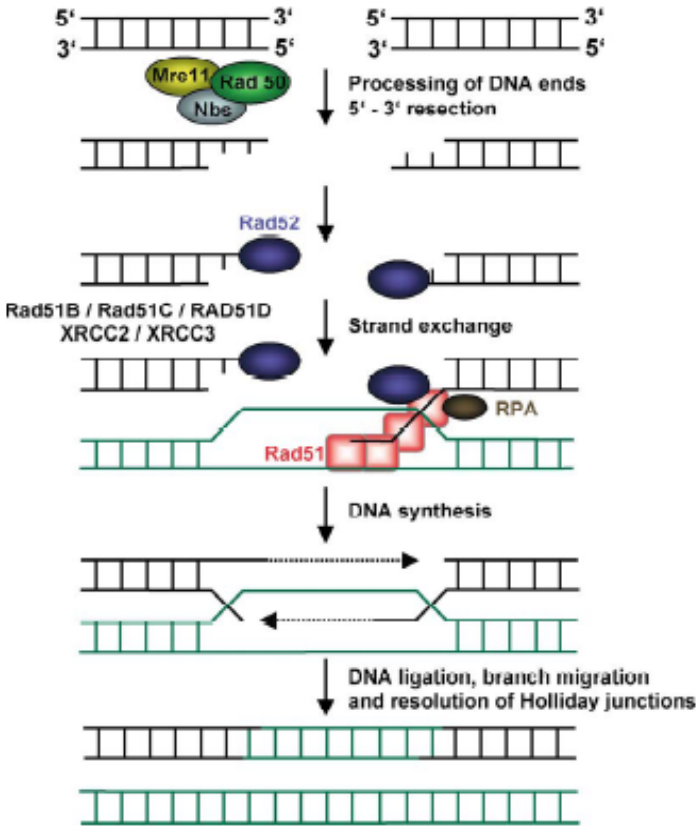

Figure 6 Homologous recombination repair pathway (*Modified from Christmann et al, 2003*).

- ***Non-homologous end-joining repair (NHEJ) pathway***

The NHEJ repair pathway is based on the direct ligation of the two double-strand-break ends and also involves numerous molecules, including LIG4 (O´Driscoll and Jeggo, 2006). During NHEJ, the two broken ends of DNA are simply pieced together, sometimes after limited processing of the DNA ends, resulting in quick, but error-prone, repair. HR is a more accurate method of repair as here information is copied from an intact homologous DNA duplex; however. As HR requires the presence of an intact sister chromatid, this method of repair can only operate in the S/G2 phases of the cell cycle in mammalian cells. NHEJ plays a major role in the elimination of DSBs during G1 phases of the cell cycle since HR is not efficient in this phase due to the lack of sister chromatids. NHEJ may be the main pathway of repair in mammalian cells especially in the G1 phase of the cell cycle. After DSB formation, the KU70/80 heterodimer binds the DNA ends. This facilitates the recruitment of DNA dependent protein kinase catalytic subunit (DNA-PKcs) to the DSB. This sequential binding of the proteins activates the phosphorylation function of DNA-PKcs, phosphorylating itself, the KU heterodimer, and other proteins involved in cell cycle regulation. It has been speculated that KU70/80 might function as an alignment factor that binds DSB ends, creating easy access for the DNA ligase IV-XRCC4 complex and increasing the efficiency and accuracy of NHEJ. The Ligase IV-XRCC4 complex then ligates the juxtaposed DNA ends. (**Figure 7**) (Agarwal et al, 2006; O´Driscoll and Jeggo, 2006).

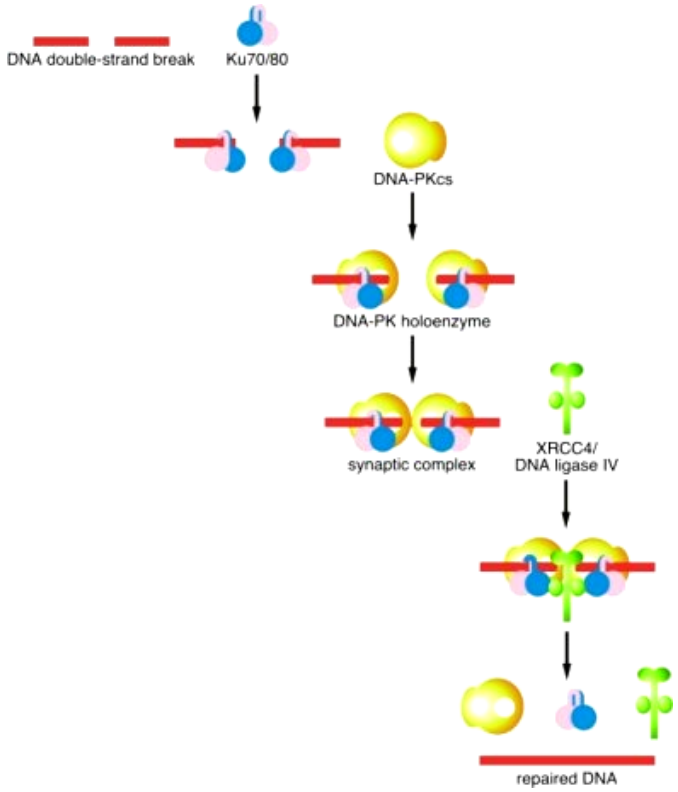

Figure 7 Non-homologous end-joining repair pathway *(Modified from O´Driscoll and Jeggo, 2006).*

Mismatch Repair (MMR)

Unintentional errors, such as base–base mismatches, insertion/deletion loops, and heterologies, can be generated during DNA replication and recombination. To prevent genetic instability that may arise from such DNA intermediates, organisms have developed the MMR system. This pathway functions to either (1) repair the mismatches or insertions/deletions formed due to polymerase infidelity/slippage or (2) prevent the occurrence of heterologous (or non-conservative) DNA exchanges (i.e. recombination). Single nucleotide mismatches or single base pair insertions/deletions are recognized primarily by the heterodimer Msh2/Msh6 (hMutSα), a complex composed of two distinct *E. coli* mutS homologs. An alternative heterodimer, Msh2/Msh3 (hMutSβ), targets insertion/deletion loops of >1, but <15, base pairs (larger loops are presumably substrates for NER). These "MutS" protein–DNA complexes then recruit the Mlh1/Pms2 heterodimer (hMutLα). Two other MutL complexes exist, i.e. Mlh1/Pms1 (hMutLβ) and Mlh1/Mlh3; a function for the former is presently unknown, while the latter is likely to contribute to the repair of insertion/deletion loops. The ensuing steps of human MMR are poorly defined, but in general, the mismatch or loop structure is

selectively excised from the newly synthesized strand of DNA and the genomic integrity restored. Enzymes found to contribute to the excision step in MMR include polymerase δ and Exo1. The PCNA-dependent polymerases (mainly polymerase δ), along with DNA ligase 1, are believed to execute repair synthesis and nick sealing, respectively, to complete the corrective process (**Figure 8**). By repairing base–base mismatches and insertion/deletion loops mistakenly introduced during DNA replication, the MMR pathway improves the fidelity of DNA synthesis 100–1000-fold (Morehnveiser, 2002; Jun et al, 2006). Defects in this system dramatically increase mutation rates, accelerating the process of oncogenesis (Hoeijmakers, 2001). Mutations in the genes of MMR have been linked to HNPCC. In particular, the mutator phenotype of individuals carrying mutations in the genes of MMR, with the vast majority occurring in *MSH2* and *MLH1*, is severe microsatellite instability, i.e. the expansion/contraction of primarily mono- and di-nucleotide repeats (Morehnveiser, 2002).

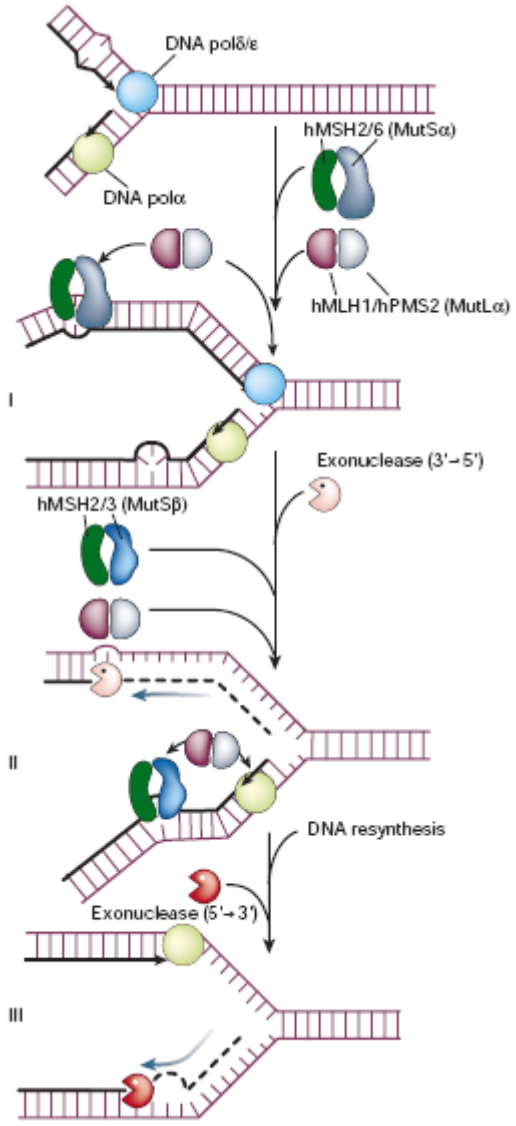

Figure 8 Mismatch repair pathway *(Modified from Hoeijmakers, 2001).*

Direct Repair (DR)

In mammals, the only known DR pathway is comprised of a single protein (and thus a single gene), termed O^6-alkylguanine (O^6-AG) DNA alkyltransferase (AGT), encoded by O^6-methylguanine (O^6-MeG) methyltransferase (*MGMT*). This protein is an ubiquitous repair protein that plays a vital role in minimizing the mutagenic effects of alkylating agents, covalently binding at O^6 position of guanine. AGT is able to act as a single protein that reverses alkylation damage (Margison and Santibanez-Koref, 2002). It transfers the alkyl group at the O^6 position of guanine to a cysteine residue within its active site, leading to the direct restoration of the natural chemical composition of DNA without the need for genomic

"reconstruction" (Gerson, 2004) (**Figure 9**). However, this repair event results in the irreversible inactivation of the AGT protein, referred to as a "suicide" reaction. AGT has been shown to remove other alkyl groups in DNA as well, such as those formed at the phosphodiester linkages in the DNA backbone and, to a lesser extent, those generated at the O^4 position of thymine. Deficiencies in *MGMT* can lead to an increase in mutations, in part because O^6-MeG mispairs with thymine during DNA replication. Overexpression of *MGMT* reduces the risk of carcinogenesis and the risk of mutations after exposure to methylating agents, instead loss of *MGMT* is associated with increased carcinogenic risk and increased sensitivity to methylating agents (Gerson, 2004).

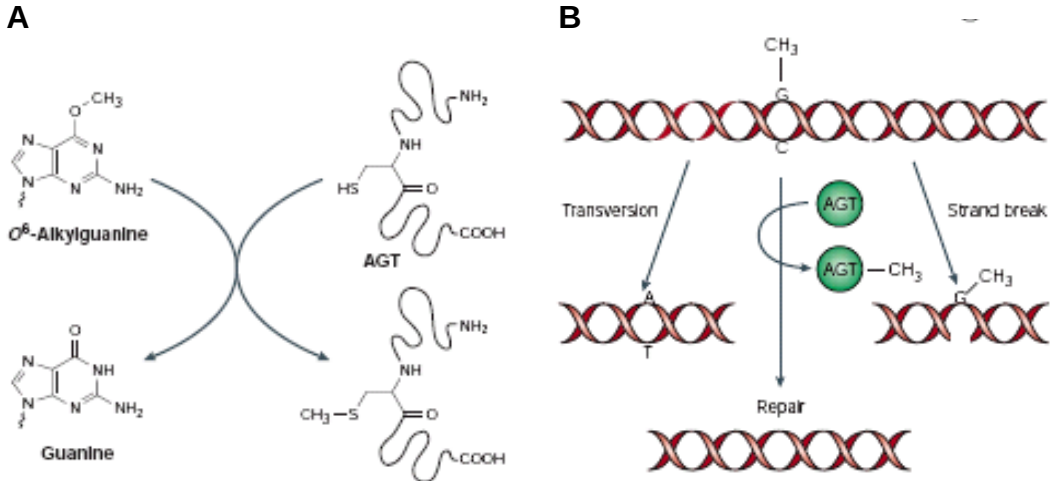

Figure 9 Direct Repair process: **A** The AGT protein reverses alkylation damage; **B** If repair of the CH_3-G lesion does not occur, a G→A transition mutation or a strand break can result *(Modified from Gerson, 2004).*

1.4 Phenotypic effect of DNA repair polymorphisms

The assessment of the individual DNA repair capacity represents a major task in the relationship to cancer etiology. The main difficulties are represented by still inadequate specific functional assays and lack of possibility to assess both intra-individual and inter-individual variability (Berwick and Vineis, 2005).

The polymorphisms of genes involved in different pathways of DNA repair may modulate the individual repair capacity in response to DNA damage, and may have an impact on individual genetic susceptibility to basically all type of cancer, including CRC (Friedberg, 2003, Spitz et al., 2003).

Over 520 amino acid substitution variants in 91 DNA repair genes have been identified in humans, while the number of other silent polymorphisms is constantly increasing, with many of them still unknown (Xi et al, 2004). An analysis of SNPs in 88 DNA repair genes and their functional evaluation, based on the conservation of amino acids among the protein family members, shows that approximately 30% of variants of DNA repair proteins are likely to affect substantially the protein function. It applies particularly for polymorphisms in *XRCC1* (Arg280His and Arg399Gln), and *XRCC3* (Thr241Met) (Savas et al., 2004). Unfortunately, for many polymorphisms the functional significance/phenotypic changes are still not experimentally shown in the general population. There are relatively few studies, sometimes based on a small number of observations and investigating few polymorphisms. Susceptibility towards ionizing radiation sensitivity, as measured by prolonged cell cycle G_2 delay, was significantly affected by amino acid substitution variants in *XRCC1* Arg194Trp, Arg399Gln and *APE1* Asn148Glu polymorphisms (Hu et al., 2001). Using the cytogenetic challenge assay, *XRCC1* 399Gln and *XRCC3* 241Met variant alleles were associated with significant increase in chromosomal deletions as compared with the corresponding homozygous wild types (Au et al., 2003). Individuals with the *XRCC1* 194ArgArg genotype exhibited significantly higher values of chromosomal breaks than those with variant Trp allele, suggesting a protective effect of this allele. On the other hand, variant Gln allele in *XRCC1* Arg399Gln was significantly associated with an increase in chromosomal breaks. These data are biologically plausible, since codon 399 is located within the *BRCA1* C-terminus functional domain and codon 194 is in the linker region of the *XRCC1* N-terminal functional domain (Wang et al., 2003). Three studies using different approaches have found a functional impact of *hOGG1* Ser326Cys polymorphism (Kohno et al., 1998, Chen et al., 2003, Yamane et al., 2004), but other studies (reviewed by Weiss et al., 2005) did not find any conclusive result for *hOGG1* genetic polymorphisms. *hOGG1* Ser326Cys polymorphism has also been found to affect the glycosylase function due to the localization and phosphorylation status (Luna et al., 2005).

In our recent study, the influence of several polymorphisms in different DNA repair pathways was investigated in association with individual DNA repair activity (i.e. the capacity to repair both γ-irradiation-specific induced- and oxidative-induced DNA damage) in a general healthy Central European population (n=244).

Irradiation-specific DNA repair rates were significantly decreased in individuals with *XRCC1* Arg399Gln homozygous variant genotype, consistent with a role of the gene in the repair pathway. In addition, the capacity to repair oxidative DNA damage was significantly decreased in individuals with *hOGG1* Ser326Cys homozygous variant genotype. The relationships between polymorphisms of *XRCC1* Arg399Gln and *APE1* Asn148Glu and functional outcomes have recently been highlighted in healthy subjects. Increasing number of variant alleles for both *XRCC1* Arg399Gln and *APE1* Asn148Glu polymorphisms resulted in a significant decrease of irradiation-specific repair rates, reflecting a gene-gene interaction (Vodicka et al, 2006). Irradiation-specific repair rates also decreased with increasing number of variant alleles in *XRCC1* Arg399Gln in combination with variant alleles for other *XRCC1* polymorphisms, Arg194Trp and Arg280His. Similarly, variant alleles of *hOGG1* Ser326Cys and *APE1* Asn148Glu exhibited a significantly decreased repair of DNA oxidative damage (Vodicka et al., 2006). The results of such tests should enable a more meaningful choice of genes/polymorphisms for association studies, though there is a still not sufficient evidence for an accurate prediction on the individual DNA repair capacity.

Many DNA repair gene variants have been studied extensively in the context of cancer susceptibility. In this context, many case-control studies have focused on identifying possible associations of SNPs in candidate DNA repair genes with altered risk for several different cancer sites. The assumption is that DNA repair is likely to work in a rather non-specific manner for different carcinogens and different cancers. Several reviews have recently tried to summarize the main results of the published studies on DNA repair genetic polymorphisms in association with cancer (Goode et al., 2002, Hung et al., 2005, Benhamou and Sarasin, 2005, Weiss et al., 2005, Manuguerra et al, 2006). However, the outcomes from epidemiological studies are not so clear. Only in the case of few genes, like *hOGG1*, *XRCC1* for BER and *XPD* for NER, consistent evidences for the association of particular genetic polymorphisms with specific cancers have been found. For instance, the variant CysCys genotype of *hOGG1* Ser326Cys polymorphism seems to be associated with an increased risk of lung cancer, esophagus, and prostate cancer (Goode et al., 2002, Hung et al., 2005, Weiss et al., 2005), and SNPs of *XPD* (Benhamou and Sarasin, 2005, Manuguerra et al., 2006) have been significantly associated with skin, breast and lung cancer. On the other hand, polymorphisms like

those of *XRCC3* gene seem to have no association with any cancer risk, despite the importance of this gene in DSB repair pathway.

1.5 DNA repair poymorphisms and susceptibility to CRC

Cancer-related genes have traditionally been classified as protooncogenes and tumor-suppressor genes. More recently, these genes have been reclassified as caretakers, gatekeepers and landscapers (Dean, 2003). Although this new classification was made in the context of high-penetrant genes and familial cancer syndromes, the concept is also applicable to low-penetrant genes and gene-environment interactions. Caretakers are responsible for maintaining genomic integrity (e.g., DNA repair, metabolic activation or detoxification), and when mutated, they increase the probability of mutation in other genes. Gatekeepers are responsible for cell cycle control, signal transduction and replication. When mutated, these genes have the ability for selective clonal expansion. Landscapers are concerned with signaling to adjacent cells (Ahmned, 2006; **Figure 3**).

The effect of food mutagens on caretaker and gatekeeper genes can theoretically be modulated by interindividual variation in the function of any enzyme that is involved in DNA damage and response (i.e., metabolic activation or detoxification, DNA repair, cell cycle control, differentiation, apoptosis, etc.).

For cancer-causing food mutagens, inter-individual variation is governed by genetic polymorphism, in which the frequency of the genetic variant in the population of interest is >1%. Sporadic cancer risk may be modified by polymorphisms in low-penetrant genes, more often in the context of exposure and not as a main effect. Although the increase in cancer risk associated with polymorphism in low-penetrant genes is small, the attributable risk in the population is large due to the high frequency of the variant (Ahmed, 2006).

Mutations in MMR genes are known to segregate in families with HNPCC (Peltomaki, 2001). However, the link between DNA repair and CRC development is even stronger as demonstrated by the association of germline mutations in *MUTYH*, a BER gene encoding a DNA glycosylase, with a predisposition to multiple colorectal adenomas and carcinomas (Al-Tassan et al., 2002).

Over the last 10 years, a growing number of studies have investigated the role of DNA repair in the CRC onset. In **Tables 1-5** are presented in summary the main

results from association studies between genetic polymorphisms in the main DNA repair pathways and risk of CRC/adenomas.

The outcomes from association studies on polymorphisms of NER genes do not show any strong and straight association with CRC risk (**Table 1**). The most frequently studied, *XPD* Lys751Gln, provided significant associations only with adenomas. In CRC development the susceptibility may play a more relevant role in the stage of adenomas, which preceeds the onset of cancer. For BER pathway, the majority of studies analyzed variants in *XRCC1* (**Table 2**).

Table 1. Association studies between genetic polymorphisms in **NER** genes and risk of CRC/adenomas

Reference	**Genes (polymorphisms)**	**Cases**	**Controls**	**Ethnicity (Country)**	**Associations**	**Interactions**
Berndt et al. 2006	*XPA* (3´UTR C→G) *XPB* (487bp 3' of STP, G→A, IVS6-108, A→C) *XPC* (Arg492His, Ala499Val, Arg687Arg, Lys939Gln) *XPD* (Lys751Gln, IVS19-70) *XPF* (Arg415Gln, Ser662Pro) *XPG* (Cys529Ser, Asp1104His) *CSB* (Met1097Val, Arg1213Gly, Arg1230Pro) *LIG1* (5´UTR C→T) *ERCC1* (Gln504Lys, 19716 C →G) *RAD23B* (Ala249Val) *RPA2* (3´UTR T→C)	250 carcinomas	2224 no history of cancer	American mixed (Caucasian 98%) (USA)	*CSB* 1097Val and 1213Gly alleles associated with ↑ CRC risk *XPC* 492His allele associated with ↑ CRC risk *XPC* haplotype containing 492His allele associated with ↑ CRC risk	SNP-SNP interaction between *CSB* 1097Val and *XPC* 492His alleles and *CSB* 1213Gly and *XPC* 492His alleles slightly ↑ CRC risk *CSB* 1097Val and 1213Gly alleles associated with ↑ CRC risk among individuals with a first-degree relative with CRC No interactions with age at diagnosis, gender, smoking habit, red meat intake, folate intake, and body mass index
Goodman et al. 2006	*XPD* (Asp312Asn) *XPF* (Arg415Gln, Glu875Gly) *XPG* (Cys529Ser, Asp1104His)	216 carcinomas (males)	255 no history of cancer (males)	Caucasian and African American (USA)	No association of single SNP	No interactions between NER (or other DNA repair) polymorphisms
Huang et al.	*XPD*	772 high-risk	777 negative	American	No association of	Smokers with *XPC* haplotype (Arg, Ala,

2006	(Asp312Asn, Lys751Gln) *XPC* (Arg492His, Ala499Val, Lys939Gln) *RAD23B* (Ala249Val) *CSB* (Met1097Val, Arg1230Pro, Gln1413Arg) *CCNH* (Val270Ala) *XPF* (Pro379Ser, Arg415Gln) *XPG* (Met254Val, Cys529Ser, Asp1104His)	adenomas	to colonscopy screening	mixed (USA)	single SNP	and Gln) associated with ↑ risk of high-risk adenomas No interactions with age, gender, and ethnicity
Moreno et al. 2006	*ERCC1* (19716 G→C, 19007 T→C, 17677 A→C, 15310 G→C, 8092 C→A) *XPD* (Asp312Asn, Lys751Gln) *XPF* (Pro379Ser, Arg415Gln) *XPG* (335 T→C)	377 carcinomas	329 hospital healthy	Caucasian (Spain)	*ERCC1* 17677C allele associated with ↑ CRC risk in an additive model ERCC1 haplotype (19716C, 19007C and 17677C) associated with ↑ CRC risk	No interactions with age
Skjelbred et al. 2006 (a)	*XPD* (Lys751Gln)	157 carcinomas 983 adenomas (227 high-risk and 756 low-risk)	399 negative to colonscopy screening	Caucasian (Norway)	*XPD* 751Gln allele associated with ↑ risk of low-risk adenoma	No interactions with smoking habit
Skjelbred et al. 2006 (b)	*ERCC1* (Asn148Asn)	156 carcinomas 981 adenomas (227 high-risk	399 negative to colonscopy screening	Caucasian (Norway)	No association of single SNP	No interactions with smoking and alcohol habits

		and 754 low-risk)				
Bigler et al. 2005	*XPD* (Asp312Asn, Lys751Gln) *XPG* (Asp1104His)	694 (384 adenomatous polyps, 191 hyperplastic polyps, 119 both types)	621 negative to colonscopy screening	Afroamerican and Caucasian (USA)	No association of single SNP Combination of *XPD* 312Asn and 751Gln alleles associated with ↑ adenoma risk *XPG* 1104HisHis genotype associated with ↓ risk of hyperplastic polyps	Heavy smokers with *XPD* combined homozygous variant genotypes or *XPG* 1104 AspAsp genotype had an ↑ risk of adenomatous polyps *XPG* 1104HisHis genotype associated with ↓ risk of hyperplastic polyps in young individuals (<60yrs) No interactions with gender, meat consumption, and alcohol and vitamin intakes
Starinsky et al. 2005	*XPD* (Lys751Gln)	456 carcinomas	87 hospital healthy	Jewish (64% Ashkenazi) (Israeli)	No association of single SNP	*XPD* 751Gln allele associated with age at diagnosis in Ashkenazi subset only
Yeh et al. 2005 (a, b) Yeh et al. 2006	*XPD* (Lys751Gln)	727 carcinomas	736 negative to colonscopy screening	Asian (Taiwan)	No association of single SNP	↑ CRC risk for combinations of *XPD, XRCC3*, and *XRCC1* genotypes with OR >1, particularly for younger individuals (<61yrs) and for rectum cases Combinations of *XPD* and *CYP1A1*2C* and *GSTT1* deletion high-risk genotypes associated with ↑ CRC risk No interactions for *XPD* polymorphism with smoking habit, alcohol and meat intake, or vegetable/fruit and fish/shrimp consumption
Mort et al. 2003	*XPD* (exon 6, exon 22, Lys751Gln) *ERCC1* exon 4 *XPG* (Asp1104His) *XPF* (Glu875Gly)	45 carcinomas	71 hospital healthy (not for all genes)	Caucasians (?) (England)	No association of single SNP	

(?) not fully specified in the study

For *XRCC1* Arg399Gln, there is a slight prevalence of studies with an increased CRC risk in association with the variant Gln allele, while for adenoma cases the same allele shows a decreased risk. A clear interpretation of the role of this polymorphism is precluded by facts that the CRC studies are conducted on different ethnic groups and, unfortunately, positive associations emerge mostly in studies with smaller sample-size. This precludes a clear interpretation of the role of this polymorphism. For two other *XRCC1* SNPs (Arg194Trp, Arg280His), significant associations have been observed only in combinations, suggesting a more relevant role of particular haplotypes rather than single SNPs. The second most frequently analysed SNP, *OGG1* Ser326Cys, provided inconclusive outcomes. The variant allele has been described in association with either increased (Goodman et al, 2006; Moreno et al, 2006) or decreased CRC risk (Hansen et al, 2005), and also in no association at all (Kim et al, 2003). Several studies analysed interactions between BER SNPs with modifiers (age, gender, smoking habits, alcohol and meat consumption). Overall, age stratification appears to be important: an influence of genetic polymorphisms seems to be more relevant in individuals with a younger age at the diagnosis (i.e. below 60 years).

No strong associations emerged for DSB gene polymorphisms from the reviewed studies (**Table 3**). *XRCC3* Thr241Met polymorphism was associated with CRC risk, but with opposite directions, as reported in (Mort et al, 2003; Krupa and Blasiak, 2004; Jin et al, 2005). Conflicting data from association studies between polymorphisms and cancer susceptibility are not unusual and often result from an insufficient sample size, as e.g. Mort et al (2003) and Krupa and Blasiak (2004) investigated far less than 100 patients. No main interactions of modifiers (i.e. smoking, alcohol, and meat consumption, fatty acids and antioxidants intake) emerge with SNPs in DSB repair genes. In the study of Jin et al. (2005), the carriers of *XRCC3* 241Met allele non-smokers and non-alcohol drinkers, showed an increased CRC risk (adjusted OR 4.85, 95% CI: 1.59–14.76 among non-smokers and adjusted OR 3.72, 95% CI: 1.48–9.39 among non-alcohol drinkers, respectively). In this case, the stratification reduced drastically the number of observations within each group. Yeh et al. (2005a, b and 2006) did not find any association for *XRCC3* Thr241Met, but a stratification of the patients for meat consumption revealed that individuals with 241ThrThr genotype and low consumers

Table 2. Association studies between genetic polymorphisms in **BER** genes and risk of CRC/adenomas

Reference	Genes (polymorphisms)	Cases	Controls	Ethnicity (Country)	Associations (Main results)	Interactions
Goodman et al. 2006	*OGG1* (Ser326Cys) *XRCC1* (Arg194Trp, Arg399Gln)	216 carcinomas (males)	255 no history of cancer (males)	Caucasian and African American (USA)	Significant P_{trend} for *OGG1* (Ser326Cys)	No interactions between BER (or other DNA repair) polymorphisms
Moreno et al. 2006	*OGG1* (Ser326Cys) *LIG3* (Lys811Thr, Arg780His) *APEX* (Gln51His, Asp148Glu) *POLB* (Pro242Arg) *XRCC1* (Arg194Trp, Arg280His, Arg399Gln) *PCNA* (1876 A→G) *MUTYH* (Tyr165Cys, Gly382Asp)	377 carcinomas	329 hospital healthy	Caucasian (Spain)	*OGG1 326C* allele associated with ↑ CRC risk *POLB* 242Arg rare allele associated with ↓ CRC risk (no homozygous variant found)	*OGG1* 326CysCys genotype associated with ↑ CRC risk in young individuals *XRCC1* 194Trp and 280His minor alleles associated with a ↓ CRC risk in young individuals
Skjelbred et al. 2006 (a)	*XRCC1* (Arg194Trp, Arg280His, Arg399Gln)	157 carcinomas 983 adenomas (227 high-risk and 756 low-risk)	399 negative to colonscopy screening	Caucasian (Norway)	*XRCC1* 280His allele associated with ↑ adenoma risk *XRCC1* 399Gln allele associated with ↓ risk in the high-risk adenoma group	No interaction with smoking habit
Hansen et al. 2005	*OGG1* (Ser326Cys)	166 carcinomas 974 adenomas	397 negative to colonscopy screening	Caucasian (Norway)	*OGG1* 326Cys allele associated with ↓ carcinoma risk	
Hong et al. 2005	*XRCC1* (Arg194Trp, Arg280His, Arg399Gln)	209 carcinomas	209 hospital healthy	Asian (South Korea)	*XRCC1* 399Gln allele associated with ↑ CRC risk	↑ CRC risk associated with alcohol intake in combined alleles 194Trp-280Arg-399Arg,

					The combined alleles *XRCC1* 194Trp-280Arg-399Gln associated with ↑ CRC risk	194Trp-280His-399Arg and 194Arg-280Arg-399Gln No interaction with smoking, dietary habits and physical activity
Stern et al. 2005	*XRCC1* (Arg194Trp, Arg399Gln)	753 adenomas	799 hospital healthy	Caucasian, African American, Latinos, Asian/Pacific Island (USA)	*XRCC1* 399GlnGln genotype associated with ↓ adenoma risk *XRCC1* 194ArgArg and 399GlnGln combined genotypes associated with ↓ adenoma risk	↑ adenoma risk associated with high monounsaturated fatty acid intake, in individuals with *XRCC1* 194ArgArg and 399GlnGln combined genotypes No interaction with polyunsaturated fatty acid intake and antioxidant intake
Yeh et al. 2005 (a, b) Yeh et al. 2006	*XRCC1* (Arg399Gln)	727 carcinomas	736 negative to colonscopy screening	Asian (Taiwan)	No association of single SNP	*XRCC1* 399Arg allele associated with ↑ CRC risk for young individuals (<61yrs) and for rectum cases ↑ CRC risk for combinations of *XRCC1*, *XPD* and *XRCC3* genotypes, particularly for younger individuals (<61yrs) and for rectum cases No interactions with smoking habit, alcohol and meat intake, or vegetable/fruit and fish/shrimp consumption
Kim et al. 2004a	*OGG1* (Arg154His)	500 carcinomas	527 hospital healthy	Asian (South Korea)	*OGG1* 154His allele associated moderate ↑ CRC risk	
Krupa et Blasiak. 2004	*XRCC1* (Arg399Gln)	51 carcinomas	100 hospital healthy	Caucasian (Poland)	*XRCC1* 399Gln allele (?) weakly associated with ↑ CRC risk	Gene-gene interaction between the *XRCC3* 241MetMet and the *XRCC1* 399ArgArg genotypes

						slightly ↑ CRC risk
Kim et al. 2003	*OGG1* (Ser326Cys)	125 carcinomas	247 cancer-free	Asian (South Korea)	No association of single SNP	*OGG1* 326CysCys genotype associated with ↑ CRC risk in group with higher meat intake. Smokers with *OGG1* 326CysCys genotype moderately associated with ↑ CRC risk No interactions with alcohol consumption, vegetable and soybean intake, physical activity and family history of cancer
Mort et al. 2003	*XRCC1*, exon 17	45 carcinomas	71 hospital healthy (not for all genes)	Caucasian (?) (England)	No association of single SNP	
Abdel-Rahman et al. 2000	*XRCC1* (Arg194Trp, Arg399Gln)	48 carcinomas	48 healthy population	(?) (Egypt)	*XRCC1* 399Gln allele associated with ↑ CRC risk	*XRCC1* 399Gln allele associated with ↑ CRC risk only in young individuals (<40yrs), and mainly in urban resident individuals

(?) not fully specified in the study

Table 3. Association studies between genetic polymorphisms in **Double-strand break repair** genes and risk of CRC/adenomas

Reference	Genes (polymorphisms)	Cases	Controls	Ethnicity (Country)	Associations (Main results)	Interactions
Goodman et al. 2006	*XRCC3* (Thr241Met) *NBS1* (Glu185Gln)	216 carcinomas (males)	255 no history of cancer (males)	Caucasian and African American (USA)	No association of single SNP	No interactions between DSB (or other DNA repair) polymorphisms
Skjelbred et al. 2006 (a)	*XRCC3* (Thr241Met)	157 carcinomas 983 adenomas (227 high-risk and 756 low-risk)	399 negative to colonscopy screening	Caucasian (Norway)	No association of single SNP	No interactions with smoking habit or alcohol consumption
Moreno et al. 2006	*XRCC2* (Arg188His) *XRCC3* (Thr241Met) *XRCC9* (Thr297Ile)	377 carcinomas	329 hospital healthy	Caucasian (Spain)	No association of single SNP	No interaction with age
Jin et al. 2005	*XRCC3* (Thr241Met)	140 carcinomas	280 cancer-free	Asian (China)	*XRCC3* 241Met allele associated with ↑ CRC risk	*XRCC3* 241Met allele in older individuals associated with ↑ CRC risk Non smokers and non using alcohol individuals with *XRCC3* 241Met allele associated with ↑ CRC risk No interaction with gender
Stern et al, 2005	*XRCC3* (Thr241Met)	753 adenomas	799 hospital healthy	Caucasian, African American, Latinos, Asian/Pacific Island (USA)	No association of single SNP	No interactions with poly and mono unsaturated fatty acids and antioxidant intake
Yeh et al.	*XRCC3*	776 carcinomas	736 negative to	Asian	No association of single SNP	*XRCC3* 241Thr allele associated

2005 (a, b) Yeh et al. 2006	(Thr241Met)		colonscopy screening	(Taiwan)		with ↑ CRC risk in low meat consumption individuals, particular in rectum cases ↑ CRC risk for combinations of XRCC3, XPD and XRCC1 genotypes with OR >1, particularly for younger individuals (<61yrs) and for rectum cases Combinations of *XRCC3* T241Met and *CYP1A1**2C high-risk genotypes associated ↑ CRC risk in women No interactions with smoking habit, alcohol intake and vegetable/fruit and fish/shrimp consumption
Krupa et Blasiak. 2004	*XRCC3* (Thr241Met)	51 carcinomas	100 hospital healthy	Caucasian (Poland)	*XRCC3* 241MetMet genotype strongly associated with ↑ CRC risk	Gene-gene interaction between *XRCC3* 241MetMet and *XRCC1* 399ArgArg genotypes slightly ↑ CRC risk
Tranah et al. 2004	*XRCC2* (Arg188His), *XRCC3* (Thr241Met, 4541 A→G, 17893 A→G)	932 adenomas	1282 cancer-free	Caucasian (?) (USA)	No association of single SNP	No interactions of alcohol and smoking habit, although both confounders increased OR of genotypes No interactions with plasma and dietary folate
Mort et al. 2003	*XRCC3* (Thr241Met)	123 carcinomas	128 hospital healthy	Caucasian (?) (England)	Moderate association of 241Thr allele with ↑ CRC	

(?) not fully specified in the study

of meat had an increased risk of CRC (OR, 2.34; 95% CI, 1.28-4.29, $P_{interaction}$=0.02). Enhanced risk was particularly pronounced in rectal cancer patients. In Yeh et al (2006), the combination of *XRCC3* Thr241Met wild type genotype and *CYP1A1*2C* variant GG genotype was associated with increased CRC risk in women (OR, 3.1, 95% CI, 1.3-7.0, P<0.01). In this case the stratification for dietary/lifestyle risk factors and gender was justified by the large size of the cohort (>700 individuals). The effect of age did not emerge, except for Jin et al (2005), where *XRCC3* 241Met allele was associated with CRC risk among older individuals (>60 years).

There are still limited data on MMR polymorphisms and CRC risk for drawing any conclusion at present (**Table 4**). Berndt et al (2007) found significant associations with *hMSH3* SNPs and Yu et al (2006) found a possible modifying effect of smoking for hyperplastic polyp risk in *hMLH1* –93A carriers. Otherwise, the studies did not reveal clearly positive associations. Interestingly, polymorphisms in *EXO1* seem to modulate inversely CRC risk, but only one study is available (Yamamoto et al, 2005) and these results should be confirmed on larger, ethnically homogeneous populations.

Four studies have analysed *MGMT* polymorphisms in relation to CRC risk (**Table 5**). The association between *MGMT* Ile143Val and Leu84Phe polymorphisms and risk of CRC was assessed in two American nested case-control studies (Tranah et al, 2006). The first population consisted of 197 women with CRC and 2,500 cancer-free women, while the second included 271 male CRC cases and 451 cancer-free men as control group. Cases were matched with controls for age and smoking history. A significant inverse association between the *MGMT* 143Val allele and CRC risk was found only in women. No association was found in the other studies on *MGMT* polymorphisms (Bigler et al, 2005; Goodman et al, 2006; Moreno et al, 2006), however, the data available are still relatively scarce, precluding any conclusion.

Table 4. Association studies between genetic polymorphisms in **Mismatch repair** genes and risk of CRC/adenomas

Reference	Genes (polymorphisms)	Cases	Controls	Ethnicity (Country)	Associations (Main results)	Interactions
Berndt et al. 2007	*hMLH1* (Ile219Val) *hMSH3* (Thr1036Ala, Arg940Gln) *hMSH6* (Gly39Glu)	237 carcinomas	2189 no history of cancer	American mixed (Caucasian 98%) (USA)	*hMSH3* 1036Ala allele associated with ↑ CRC risk *hMSH6* 39GluGlu genotype associated with ↑ risk of rectal cancer *hMSH3* haplotype containing both 940Gln and 1036Ala alleles associated with ↑ CRC risk	*hMSH3* 1036Ala allele associated with ↑ risk of CRC in interaction with meat intake ≥10 g/day *hMSH3* haplotype containing 1036Ala allele associated with ↑ CRC risk among individuals with meat intake ≥10 g/day No interactions with gender, smoking habit, folate intake, alcohol consumption, and family history
Yu et al. 2006	*hMLH1* (-93G→A, Ile219Val) *hMSH6* (Gly39Glu)	719 (401 adenomas,195 hyperplastic polyps, 123 both types)	624 negative to colonscopy screening	Caucasian-American (97%) (USA)	No association of single SNP	*hMLH1*-93 A allele is associated with ↑ risk of hyperplastic polyps associated with smoking.
Yamamoto et al. 2005	*EXO1* (Thr439Met, Pro757Leu)	102 carcinomas	110 healthy population	Asian (Japan)	*EXO1* 439Met allele associated with ↑ CRC risk *EXO1* 757 LeuLeu genotype associated with ↓ CRC risk	*EXO1* 439MetMet and 439ThrMet, with *EXO1* 757ProLeu genotypes are associated with ↑ CRC risk
Kim et al. 2004b	*hMLH1* (Ile219Val, Val384Asp) *hMSH2* (Leu390Phe, gIVS12)	107 carcinomas	330 healthy controls and 107 1st degree relatives of cases	Asian (Korea)	No association of single SNP	
Peterlongo et al. 2003	*hMSH6* (Val509Ala, -159 C→T)	167 carcinomas	190 healthy controls	American Jews (USA)	No association of single SNP	

Table 5. Association studies between genetic polymorphisms in *MGMT* gene (**Direct repair)** and risk of CRC/adenomas

Reference	Genes (polymorphisms)	Cases	Controls	Ethnicity (Country)	Associations (Main results)	Interactions
Goodman et al. 2006	*MGMT* (171 C→T)	216 carcinomas (males)	255 no history of cancer (males)	Caucasian and African American (USA)	No association of single SNP	No interactions between MGMT and other DNA repair SNPs
Moreno et al. 2006	*MGMT* (171 C→T, Leu84Phe, Ile143Val)	377 carcinomas	329 hospital healthy	Caucasian (Spain)	No association of single SNP	No interactions with age
Bigler et al. 2005	*MGMT* (Leu84Phe, Ile143Val)	694 (384 adenomatous polyps, 191 hyperplastic polyps, 119 both types)	601 negative to colonscopy screening	African American and Caucasian (USA)	No association of single SNP	*MGMT* combined genotypes showed an interaction with smoking habit No interactions with age, gender, meat consumption, and alcohol and vitamin intake
Tranah et al. 2006	*MGMT* (Leu84Phe, Ile143Val)	197 carcinomas (females) 451 carcinomas (males)	2500 cancer-free (females) 451 cancer-free (males)	Caucasian-American (97%) (USA)	*MGMT* 143Val allele associated with ↓ risk of CRC in cohort of women No association of single SNP in men cohort	*MGMT* 84Phe allele associated with ↑ risk of CRC among women consuming ≥0.5 drink/day. No interaction between alcohol intake and *MGMT* Ile143Val SNP *MGMT* 84Phe and 143Val alleles associated with ↓ risk of CRC among women with BMI ≥25 *MGMT* 84LeuLeu genotype and use of postmenopausal hormone associated with ↑ risk of CRC No interactions with smoking habit, folate, and processed meat intake in women. No interactions with BMI, alcohol consumption, and smoking history in men

1.6 Pharmacogenomics and CRC: prognosis and individual susceptibility.

The importance of individual susceptibility is most likely not limited only to the onset of CRC, but involves also prognosis and efficacy of chemotherapy and radiation therapy. The prognosis of a patient with CRC is highly impacted by various factors at the time of diagnosis, such as localization of the tumor, quality of surgical procedures, gender, age, and patient's overall performance status (Stoehlmacher and Lenz, 2003). In addition to the clinical/pathological staging (a major factor for the success of surgical operations, postoperative management and survival expectancies), the possibility to identify cancer patients with high likelihood of recurrence, or experiencing clinical toxicity, has a significant impact on the development of more efficient/less toxic treatment strategies. Inter-individual variations in response and toxicity to a particular therapy may be due to genetic alterations in drug targets, metabolizing enzymes, efflux and DNA repair systems at the genomic, mRNA and protein levels. Thus, the main aim of pharmacogenetics screening before treatment is to identify patients who may respond to particular chemotherapeutic agents and patients who may encounter increased toxicity to the same agents on the base of patient's genetic information (Lenz, 2003, Russo et al., 2005).

Determination of genetic polymorphisms is becoming important as a possible method for helping the oncologist to decide on a more specific, personalized therapeutic approach and to provide crucial information for drug development. Currently, only few promising polymorphisms have been identified, or at least tested, for chemotherapy success and toxicity in CRC treatment. The most studied polymorphisms are in the thymidilate synthase (TS) gene, which is the main target of chemotherapeutic agents such as 5-fluorouracil and capecitabine, widely used for CRC treatment (Stoehlmacher et al., 2003). DNA repair genetic polymorphisms have also been investigated in the case of treatments based on platinum agents, cisplatin and oxaliplatin, because an increased DNA repair capacity plays an important role in chemoresistance to platinum-based compounds (Lenz, 2003). DNA adducts caused by the bulky 1,2-diamino-cyclohexane ring containing oxaliplatin are considered to mediate increased cytotoxicity and more effectively block DNA replication in comparison to other platinum agents. Oxaliplatin-based therapy, in particular, has been shown to be successful in concomitance with other

agents like 5-fluorouracil (Stoehlmacher et al., 2003). Enzymes of NER pathway are thought to repair DNA damage caused by platinum agents and several studies demonstrated the inverse relationship between impaired DNA repair capacity and increased response rates to platinum drugs (Kweekel et al., 2005).

Three studies addressed the possible modulating role of different DNA repair polymorphisms in relation to the response to 5-fluorouracil/oxaliplatin treatments. In 73 patients with metastatic CRC, three common *XPD* polymorphisms (Cys156Ala, Asp312Asn, and Lys751Gln) were investigated for their possible impact on the outcome of the therapy (Park et al., 2001). Among those tested for the Lys751Gln polymorphism, 24% (5 of 21) patients with the 751LysLys genotype responded, versus 10% (4 of 39) and 10% (1 of 10) of those with the 751LysGln and 751GlnGln genotypes (P = 0.015). The median survival for those with the 751LysLys genotype was 17.4 (95% CI 7.9-26.5) versus 12.8 (95% CI 8.5-25.9) and 3.3 (95% CI 1.4-6.5) months for patients with the heterozygous and homozygous variant genotype, respectively (P=0.002). The other two investigated *XPD* polymorphisms were neither associated with any response to 5-fluorouracil/oxaliplatin nor with survival. Stoehlmacher et al (2001), in 61 patients (same therapy as above) observed that individuals with at least one Gln variant allele in *XRCC1* Arg399Gln polymorphism were at increased risk of chemotherapy failure. The role of two polymorphisms in the *ERCC1* gene (in codon 118 and in 3'-untranslated region) was recently evaluated for the clinical outcome to platinum-based chemotherapy in 106 patients with advanced refractory CRC (Park et al. 2003). *ERCC1* codon 118 SNP causes a C→T substitution, but codes for the same amino acid, asparagine, and may be associated with different *ERCC1* gene expression. *ERCC1* 3'-untranslated region may affect mRNA stability. The authors found a significant association between the *ERCC1* codon 118 polymorphism and clinical outcome: patients with the CC genotype had a median survival of 15.3 months (95% CI, 6.0-12.1), whereas 11.1 months (95% CI, 5.8-16.2) for those with CT and TT genotypes. The *ERCC1* codon 118 polymorphism may be a useful predictor of clinical outcome in advanced CRC cases. Viguier et al. (2005), analyzed 91 patients treated for metastatic CRC in a retrospective study, and observed a higher response to combined chemotherapy of oxaliplatin/5-fluorouracil in individuals with variant T allele in *ERCC1* codon 118 polymorphism. No significant differences were detected when patients were treated only with 5-FU or

with 5-FU and irinotecan. Shirota and collaborators (2001), have shown that CRC patients with high *ERCC1* gene expression levels, treated with 5-FU/oxaliplatin, exhibited shorter surviving period than those with low expression levels. In the recent study, using univariate analysis, adjusted for age, sex, and Duke's stage, *ERCC1* 19007T>C was associated with worse prognosis of CRC (hazard ratio-HR, 1.51; 95% CI, 1.01-2.27), while polymorphisms in *XRCC1* Arg399Gln (HR, 0.38; 95% CI, 0.17-0.85), *XRCC3* Thr141Met (HR, 0.66; 95% CI, 0.45-0.97), and *MGMT* Leu84Phe (HR, 0.14; 95% CI, 0.02-0.99) were significantly associated with better prognosis. These associations were stronger among patients receiving adjuvant chemotherapy (Moreno et al. 2006).

Gordon et al. (2006) selected 21 polymorphisms in 18 genes relevant to CRC (cell cycle regulation, drug metabolism, tumor microenvironment and *ERCC1*, *XRCC3*, *APE1* and *RAD51* genes for DNA repair) and investigated the risk of tumor recurrence in a total of 90 patients treated with chemotherapy (5-FU) combined with radiotherapy (pelvic radiation). None of the investigated DNA repair polymorphisms affected the risk of recurrence, while a polymorphism in IL8, an inflammatory cytokine with angiogenic potential, seemed to modulate recurrence significantly.

Although the studies and the outcomes are still scarce, it is becoming more and more evident that the ultimate goal of a therapy should be to use those anticancer drugs that are based on the profile of a particular patient’s tumor, in order to maximize the potential response to therapy (Lenz, 2003).

1.7 Cell cycle genes

The cell cycle comprises a series of tightly controlled events that drive the replication of DNA and cell division. It is divided into several phases: preparation for (G1 phase), and execution of, DNA synthesis (S phase), a second gap phase (G2), and mitosis (M). Quiescence (G0) is a biochemically distinct state from which cells can re-enter the cell cycle and go on to DNA replication and mitosis. The transitions between these phases are regulated by changes in the activity of specific cyclin-dependent kinases (CDKs), with Cdk1/Cdk2 and Cdk2/Cdk4/Cdk6 controlling the transitions from G2 to mitosis and G1 to S phase, respectively. CDK proteins generally remain at constant levels throughout the cell cycle, while binding partners (such as cyclins) and post-translational modifiers (including kinases and phosphatases) undergo periodic fluctuations to regulate DNA synthesis and cell division. The sequential accumulation of different cyclins allows the formation of specific cyclin–CDK complexes that target substrates involved in transitions between the cell cycle phases (Caldon et al, 2006; **Figure 10**).

Deregulation of cell cycle and cell proliferation mechanisms have an important role in carcinogenesis. A number of cell cycle genes, such as cyclins, CDKs, and the regulators of the CDKs, are found frequently mutated in many types of cancer. In addition, germ-line mutations in several cell cycle control genes, such as *RB1*, *3; BRCA1* and *BRCA2*, *4; TP53*, *5; NF2*, *6,* and *CHEK27* have been found to cause strong genetic predisposition to cancer in individuals. Although control of the G2/M transition is implicated in events in cancer resulting in chromosomal aberrations, the G1/S transition encompasses many of the important cell cycle events that may be specifically altered in CRC, including the actions of the oncogenes/tumor suppressors cyclin E, cyclin D1, and p27 (Caldon et al, 2006).

In particular, abnormal expression of regulatory proteins that control G1/S phase transition, a critical rate-limiting step in cell cycle progression, are frequently observed. G1/S transition requires phosphorylation of the retinoblastoma protein (pRb), which results in the release of the E2F family of transcription factors that in turn activate genes essential for entry into S phase. Phosphorylation of pRb is initiated by cyclin D1/(CDK)4-6 complexes and completed by cyclin E/CDK2 in late G1. Alterations in cyclins and/or CDKs expression result in increased cell proliferation and are thought to contribute to malignancy. Furthermore, down-

regulation or inactivation of the CDK inhibitors, including p21Waf1/Cip1, p27Kip1, and p16Ink4a, which normally cause G1 arrest by binding to cyclin-CDK complexes, are often observed in diverse human tumors, further rendering the cell susceptible to uncontrolled extracellular proliferation signals. Frequently mutated in a wide range of human cancers, p53 is a negative regulator of cell cycle control, which inhibits cell cycle progression in part by activating p21Waf1/Cip1 expression, and also controls the exit of cells from the cell cycle into programmed cell death (Bali et al, 2004).

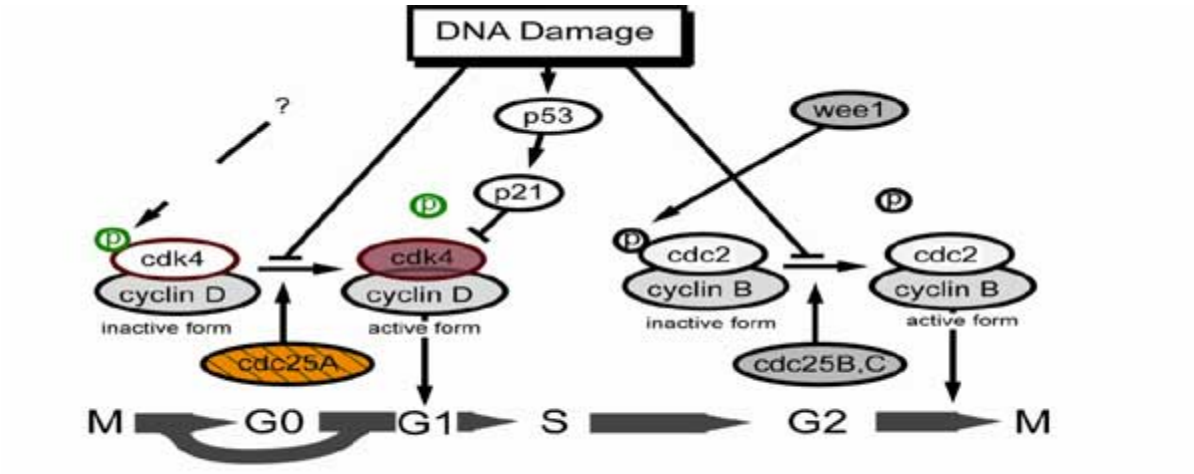

Figure 10 Simplified schema of DNA damaged induced cell cycle control (*Modified from Caldon et al, 2006*)

TP53

TP53 represents one of the most studied tumor suppressor genes in biology. Its product, the p53 protein, is referred to as 'the guardian of the genome', and represents a key regulator of cellular growth control (Pietsch et al 2006). p53 is a 53 kDa phosphoprotein, encoded by 393 amino acids forming five highly conserved regions and four functional domains (Harris and Hollstein, 1993). In response to a variety of stress signals, (including genotoxic stress, and oncogene activation) the p53 protein is post-translationally stabilized, leading to its activation as a sequence-specific transcription factor. This stabilization can then lead to different programs, depending on the cell of origin or cellular context. These include cell cycle arrest, senescence, or apoptosis (Jin and Levine, 2001). As a tumor suppressor, p53 is essential for preventing inappropriate cell proliferation and maintaining genome integrity in relation to genotoxic stress. Following various intracellular and

extracellular stimuli, such as DNA damage (induced by ionizing radiation, UV radiation, application of cytotoxic drugs or chemotherapeutic agents, and infectious virus), heat shock, hypoxia, and oncogene overexpression, wild type p53 is activated and emerges as a pivotal regulatory protein which triggers diverse biological responses, both at the level of a single cell as well as in the whole organism. p53 activation involves an increase in overall p53 protein level as well as qualitative changes in the protein through extensive post-translational modification, thus resulting in activation of p53-targeted genes. For example, in response to DSBs in DNA damage, ATM protein kinase is activated which in turn activates Chk2 kinase. Both ATM and Chk2 then both phosphorylate p53 at distinct sites leading to p53-dependent cell cycle arrest or apoptosis (Bai and Zhu, 2006).

Among various cellular responses induced by p53, most notable are the induction of cell cycle arrest and apoptosis. It appears that the ability of p53 to prevent cell growth is pivotal to its tumor suppressor functions. p53 can induce cell cycle arrest in the G1, G2 and S phases of the cell cycle. The induction of cell cycle arrest at G1 and G2 by p53 provides additional time for the cell to repair genomic damage before entering the critical stages of DNA synthesis and mitosis. The arrested cells can be released back into the proliferating pool through p53's biochemical functions that facilitate DNA repair including NER and BER (See **Figure 11**, Bai and Zhu, 2006).

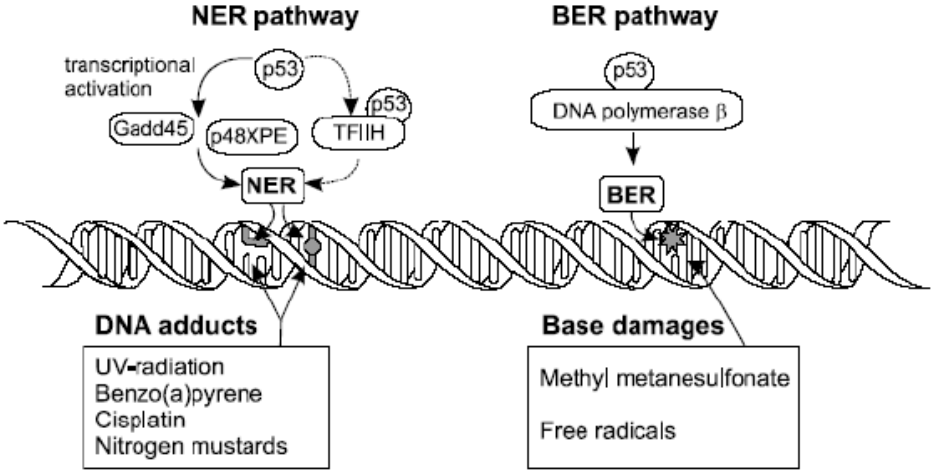

Figure 11 p53's biochemical functions may facilitate DNA repair including NER and BER pathways *(Modified from Bai and Zhu, 2006*).

p53 is well-known as the most frequently mutated gene (in more than 50% of human tumors) in human cancer. Some of these mutations have already been correlated to specific clinical phenotypes. It is therefore conceivable that the existence of natural variants of p53 could be linked with the development of specific diseases, owing to differences in the activity of variant proteins in this pathway, and could then represent an interesting predictive marker for preventive and early intervention strategies. The natural genetic variants of p53 have thus emerged as a resource to be studied in the understanding of inter-individual differences in cancer risk and therapy.

Several polymorphisms have been identified in the *TP53* gene (Olivier et al., 2002). Most of these polymorphisms are SNPs affecting a single base. A great number of these natural variants are localized in non-coding regions of the gene (introns). Among the polymorphisms found in the coding regions (exons) of *TP53*, only two alter the amino-acid sequence of its product, proline (Pro) to serine (Ser) at residue 47, and arginine (Arg) to proline (Pro) at residue 72.

The codon 72 polymorphism (TP53 Arg72Pro)

This common SNP results in a non-conservative change of an Arg to a Pro at amino acid 72 that results in a structural change of the protein giving rise to variants of distinct electrophoretic mobility (Harris et al., 1986; Matlashewski et al., 1987). This polymorphism occurs in a proline-rich region of p53, which is known to be important for the growth suppression and apoptotic functions of this protein (Walker and Levine, 1996; Sakamuro et al., 1997). Beckman and co-workers (1994) demonstrate that the frequency of the Pro72 allele differs with latitude, increasing in a linear manner as populations near the equator. These observations led the authors to suggest that the codon 72 variants differed in biological activity, and further that these differences in activity might be subject to selection in areas of high ultraviolet light exposure.

The Arg72 and Pro72 isoforms of p53 differ from the biochemical and biological point of view. Arg72 variant of p53, when in cis with certain tumor-derived mutations, might have enhanced tumor suppressive function owing to increased ability to inactivate p73 (the p53-homolog). Subsequent studies suggest that the ability of Arg72 to target and inhibit p73 may be cell-type dependent (Vikhanskaya et al., 2005). Specifically, these authors demonstrated that some of the p53 tumor

derived mutants that are unable to bind and inhibit p73, are still able to confer resistance to drug treatment. This result may suggest that Arg72-containing mutants may possess other mechanisms to disrupt chemotherapy-induced apoptosis.

In non-mutated forms of p53, the Arg72 variant has a significantly increased ability to induce programmed cell death, in cells containing inducible versions of p53, as well as in cells homozygous for Arg72 and Pro72 (Bonafe et al., 2002; Dumont et al., 2003).

In summary, the combined data from several groups has confirmed the altered apoptotic potential of the codon 72 polymorphic variants, with the Arg72 variant demonstrating enhanced apoptotic ability, and the Pro72 variant demonstrating enhanced growth arrest (Pim and Banks, 2004). Based on these findings, a number of studies have tried to establish a correlation between the *TP53* Arg72Pro polymorphism and the risk to develop certain types of cancer. In general, these studies have not yielded consistent results. This may be accounted for by the fact that the Arg72 allele, when found in mutant forms of p53, might be predicted to enhance tumor development (increased inactivation of p73). However, when found in the context of wild-type p53, it might be predicted to better inhibit tumor development (increased apoptotic ability).

The IARC TP53 Mutation Database (www.iarc.fr/P53/) lists 15 common polymorphisms in the non-coding region of *TP53*. Some of these natural variations have also been associated with increased risk of cancer development, although in the absence of clear indications that such variants alter the function of p53, it remains possible that these findings are the result of linkage to other, functionally significant, polymorphisms of p53.

Polymorphism of intron 3 (PIN3)

Among all of the polymorphisms identified in the *TP53* gene, the polymorphism in intron 3 (PIN3, A1 allele is the wildtype and allele A2 is referred to a 16 bp duplication) has also been frequently studied. However, only a single work has demonstrated an altered activity of this natural variant. Harboring the assumption that the PIN3 A2 variant allele might influence alternative splicing of p53, Gemignani et al. (2004) instead reported a reduced amount of steadystate RNA for this allele in immortalized lymphoblastoid cell lines, relative to wild type. These

results were re-capitulated with mRNA extracted directly from patient lymphocytes. Other investigators have reported that the A2 variant allele is associated with decreased apoptotic and DNA repair capacity in lymphoblastoid cell lines (Wu et al., 2002). Consistent with these altered functional activities, several studies have correlated the intron 3 duplication with an increased risk of various cancers, including cancer of the colon (Gemignani et al., 2004), lung (Wu et al., 2002), breast (Weston and Godbold, 1997; Wang-Gohrke et al., 1999; Powell et al., 2002), and ovary (Runnebaum et al., 1995; Wang- Gohrke et al., 1999). However, other groups have failed to confirm these results (Khaliq et al., 2000; Mitra et al., 2005). Analysis of haplotypes remains a much more powerful approach than single polymorphism investigations, since the integration of an increasing number of common genetic variations in the analysis should implement an increased statistical power in such studies.

Functionally significant polymorphisms in the p53 pathway exist which may impair the function of this pathway. In some cases, these variants are clearly associated with altered age of onset of cancer and its prognosis. However, whether these variants are associated with altered cancer risk is not currently clear. Such associations await combined analyses of multiple variants in this pathway, along with more precise functional studies.

Cyclin D1 (CCND1)

Cyclin D1 gene (also known as *CCND1*) is involved both in normal regulation of the cell cycle and in neoplasia, where it is frequently overexpressed. *CCND1* plays an important role in the transition from the G1 phase to the S phase of the cell cycle. Amplification or overexpression of the *CCND1* gene is common in a variety of cancers, and induces proliferation (Ahmed, 2006). *CCND1* harbors the capacity to modulate cell cycle progression. A wealth of studies systematically defined the manner in which *CCND1* functions to regulate cell cycle and oncogenic transformation in mammalian cells. The *CCND1* locus is known to be amplified in specific tumor types and it is thought that this event determines a net increase in the proto-oncogenic functions of the cyclin D1 protein (Knudsen et al., 2006).

CCND1 expression is highly regulated, as would be expected based on its powerful role in proliferative control. Its expression is induced as a delayed early response to

many mitogenic signals, and is universally associated with the transition from quiescence into the proliferative cycle (reviewed by Knudsen et al., 2006). Among the cyclins that regulate G1 progression, it is hypothesized that stimulation of *CCND1* expression represents the point at which mitogenic signal transduction cascades are integrated to mediate engagement of the cell cycle machinery (**Figure 10**).

After induction, both CCND1 mRNA and protein levels are under stringent regulation. First, *CCND1* mRNA levels are dramatically increased following mitogenic stimulation. Additionally, many oncogenes (e.g. *Ras* and *b-catenin*) harbor the capacity to induce CCND1 promoter activity (Tetsu and McCormick, 1999). Once produced, the CCND1 protein is intrinsically unstable, thus providing an additional level of regulation. Cell cycle progression reflects the induction of a threshold of CCND1 protein, which is determined by the rate of expression versus destruction. Lastly, CCND1 is marked for degradation after genomic insult (Agami and Bernards, 2000), thus preventing cell cycle progression in the presence of DNA damage. Combined, these mechanisms contribute to the accumulation of CCND1 protein and are believed to be critical for ensuring the progression into the cell cycle is restricted to the appropriate mitogenic context.

Over 100 single nucleotide polymorphisms have been identified spanning the *CCND1* locus and catalogued in public single nucleotide polymorphism databases (dbSNP: www.ncbi.nlm.nih.gov/SNP/; HapMap: www.hapmap.org; or GeneSNPs: www.genome.utah.edu/genesnps/). Of the polymorphisms identified, the *CCND1* G/A870 polymorphism has received the most investigation. The polymorphism frequency in the Caucasian population is approximately 44% A and 56% G, depending on the study (Simpson et al., 2001; Sanyal et al., 2004), but large variances between racial and ethnic groups have been reported. Owing to the significance of *CCND1* in human cancer, a large number of epidemiological studies have challenged the influence of this particular polymorphism in cancer susceptibility and disease outcome. These studies generally compare the allelic frequency of G/A870 in disease affected or unaffected individuals, and assess correlations with clinical parameters (e.g. stage at diagnosis or overall survival).

The majority of studies link the A-allele to increased cancer risk and poor disease outcome, with the largest associations observed with the A/A genotype. In these

studies, relative risks were significant but typically modest, with many studies reporting less than a two-fold effect. Such a result is in part to be expected for a common allele (i.e. A-870) that contributes to a complex phenotype. However, results have been inconsistent and some studies have implicated the G-allele with increased cancer risk, and others have ascribed no significant value to any allele of the G/A870 polymorphism. Combined, these results indicate that individual alleles may harbor differential effects in distinct tumor types. However, even within a specific tumor type (e.g. CRC) there have been disparate conclusions regarding the significance of the polymorphism. These disparities could be reflective of the patient population under study and the possible involvement of external factors (e.g. smoking or obesity) that have been suggested to cooperate with the polymorphism in specific studies (Buch et al., 2005; Shu et al., 2005). Important issues that remain to be resolved are whether the G/A870 polymorphism is the specific causal variant and whether there are other polymorphisms at this locus that are biologically relevant. It is formally possible that G/A870 is a proxy that is in linkage disequilibrium with the actual functional variant that is modulating cancer risk. Such a possibility may in part explain some of the discrepancies associated with the role of G/A870 in cancer in different study populations and emphasizes the importance of conducting linkage disequilibrium or haplotype-based investigations of common genetic variation across the entire locus. One group has coordinately analysed both the G/A870 and the G/C1722 polymorphism of *CCND1* (Holley et al., 2001). This study indicated that the two polymorphisms are in linkage disequilibrium, such that individuals harboring the A870-allele are most likely also carrying the C1722-allele. In spite of this observation, each polymorphism had a distinct influence on disease (Holley et al., 2001), suggesting that other variants in addition to the G/A870 variant in *CCND1* may be important. Therefore, although more exhaustive studies will be required to conclusively determine the involvement of the G/A870 polymorphism in cancer, there is significant evidence that it alters risk, thus necessitating study of the functional significance of the polymorphism (reviewed by Knudsen et al, 2006).

1.8 Cell cycle gene poymorphisms and susceptibility to CRC

The *TP53* gene plays a fundamental role in preventing the replication of damaged DNA (Sengupta and Harris, 2005). Somatic mutations in the *TP53* gene have been found in many tumor types, including CRC (Ilyas and Tomlinson, 1996). Three different *TP53* polymorphisms (*i.e.,* in intron 3 and 6, and Arg72Pro polymorphism in exon 4) have been studied in sporadic CRC patients. The Arg72Pro polymorphism appears to be functionally relevant because the wild type allele (Arg) has a weaker affinity for several transcription-activating factors *in vitro* (Thomas et al., 1999). *TP53* Arg72Pro is also the most frequently investigated polymorphism in association studies on CRC risk, although over all, inconsistent results have been so far obtained (Olschwang et al., 1991, Kawajiri et al., 1993, Sjalander et al., 1995, Gemignani et al., 2004). Two studies (Kawajiri et al., 1993, Gemignani et al., 2004) have suggested an increased risk associated with the Pro carrier genotypes, while in other studies the same genotypes have been associated with a null or statistically nonsignificant inverse association. Recently, Koushik et al., (2006) have found an association of Arg72Pro polymorphisms with increased risk of adenoma, but not with risk of CRC, except for women. This suggests that this polymorphism may play a role in the early stages of colorectal neoplasia and possibly in progression to invasive disease, depending on site and sex. Although the functional significance of the other two polymorphisms is unclear, intronic sequences in *TP53* have been implicated in the regulation of gene expression and in DNA protein interactions (Avigad et al., 1997). Interesting associations have been found for the PIN3 polymorphism: carriers for variant allele (both heterozygous and homozygous) showed a decreased CRC risk (Sjalander et al., 1995, Gemignani et al., 2004).

CCND1 is also a key cell cycle regulatory protein, the expression and subcellular localization of which is often altered in human tumor cells. A common A/G single nucleotide polymorphism (G/A870) in exon 4 of the *CCND1* gene is associated with the presence of 2 distinct mRNA transcripts for this G1/S regulatory protein (Sawa et al., 1998). This polymorphism could act both as a modifier of phenotypic expression in inherited CRC and as a low-risk susceptibility factor in sporadic cancer. Although still no clear and unambiguous conclusions can be drawn so far, several studies have observed an association between A variant of this polymorphism and an increased risk of both adenomas (Lewis et al., 2003) and CRC

(Kong et al., 2001, Porter et al., 2002, Le Marchand et al., 2003, Jiang et al., 2006), although in different ethnic groups. On the contrary, three studies did not find any association (Grieu et al., 2003, Schernhammer et al., 2006, Probs-Hensch et al., 2006), and one observed an inverse association with the G allele (Hong et al., 2005b).

2. Aim of the study

The aim of the present work is to investigate the associations between the risk of CRC and **1)** polymorphisms in genes involved in main DNA repair pathways (NER, BER, and DSB), **2)** polymorphisms in the cell cycle control genes (*CCND1*, *TP53*) in a hospital-based case-control study. Potential interactions between studied polymorphisms and most important confounders (such as age and smoking) are also investigated. 532 CRC cases and 532 age- and sex-matched controls have been selected from a large cohort collected in the Czech Republic in the last four years.

Rationale for the topic of PhD Thesis is based on following assumptions: **A)** CRC represents a serious health problem in the Czech Republic, as the incidence ranks the third highest worldwide and the incidence of rectal cancer is the highest. PhD Thesis is a part of an extensive project aimed at the evaluation of genetic factors involved in the onset of CRC. **B)** DNA repair gene polymorphisms were selected, assuming that environmental/life style factors play important role in the sporadic CRC risk, probably due to the accumulation of DNA damage over years. The relationship between the DNA repair genotypes (in particular for base excision repair) and the functional outcome recently investigated the cancer-free population from the same region has provided the necessary background for the selection of the investigated SNPs. Additionally, our previous work revealed an association between chromosomal damage and polymorphisms in NER genes. **C)** The close link between DNA repair pathways and cell cycle control inspired us to address SNPs in genes involved in double-strand break repair and cell cycle control (e.g. *NBS1-TP53*). **The experimental work** of the present PhD Thesis was performed in the Department of Molecular Biology of Cancer (Institute of Experimental Medicine, Academy of Science of the Czech Republic, Prague).

The PhD Thesis presents the results on the association between CRC risk and selected polymorphisms in DNA repair genes (*XPD* Lys751Gln, *XPG* Asn1104His, *XPC* Lys939Gln, *XRCC1*, Arg194Trp and Arg399Gln, *hOGG1* Ser326Cys, *APE1* Asn148Glu, *XRCC3* Thr241Met and *NBS1* Glu185Gln) and in cell cycle control genes (*CCND1* G/A870, *TP53* PIN3 and Arg72Pro).

3. Material and methods

3.1 Study population

For the present hospital-based case–control study six oncological and five large gastroenterological departments all over the Czech Republic contributed by providing blood samples and anamnestical data. The study is based on incident cases, the recruitment of which started in September 2004 and finished in February 2006.

Cases consist of patients with positive colonoscopic results for malignancy, histologically confirmed as colon or rectal carcinomas. Controls were defined as subjects undergoing colonoscopy for various gastrointestinal complaints and sampled at the same time as cases, whose colonoscopic results were negative for malignancy or idiopathic bowel diseases.

Study subjects provided the information on their lifestyle habits (smoking, drinking, diet etc.), tentative occupational exposure to xenobiotics, and family/personal history of cancer, with the use of structured questionnaires.

Blood samples were collected from 884 CRC patients and 598 controls. Subsequently, 532 case control pairs were created, matching strictly for age and sex.

The genetic analyses did not interfere with diagnostic or therapeutic procedures for the subjects. DNA was isolated from coded blood samples and stored at –80°C.

All participants signed an informed written consent and the design of the study was approved by the Ethical Committee of the Institute of Experimental Medicine, Prague, Czech Republic.

3.2 Genotypes analyses

DNA repair genotyping

SNPs in genes encoding various DNA repair enzymes were determined by PCR-RFLP or by Real-Time PCR allelic discrimination assay. For *XPD* Lys751Gln, *XPG* Asn1104His, *XPC* Lys939Gln, *XRCC1*, Arg194Trp and Arg399Gln, *hOGG1* Ser326Cys, *XRCC3* Thr241Met, the PCR was carried out using primers and conditions given in Vodicka et al (2004, 2006). The amplified fragments were digested with appropriate restriction endonucleases and the digested PCR products

were resolved on 2% agarose gel and visualized under UV light after staining with ethidium bromide. Genetic polymorphisms in *APE1* Asn148Glu and *NBS1* Glu185Gln were analysed using the TaqMan allelic discrimination assay (Applied Biosystems, Assay-on-demand, SNP Genotyping products: C_26470398_10 for *NBS1* and C_8921503_10 for *APE1*). The TaqMan genotyping reaction was amplified on a 7500 Real-Time PCR system (95°C for 10 min, 92°C for 15 sec, and 60°C for 1 min for 40 cycles).

Cell cycle genes genotyping

SNPs in genes encoding cell-cycle control enzymes were determined by PCR-RFLPanalysis. For *TP53* PIN3 polymorphism the PCR was carried out using primers and conditions given in Gemignani et al (2004). The167 bp *CCND1* G/A870 PCR products were amplified with the primers 5′-GTGTAAGTTCATTTCCAATCCGC-3′(sense) and 5′-TAAGTGAGGGTGATGTCCC-3′(antisense) and digested with *MspI*. The G allele revealed 145 and 22 bp fragments following digestion and 2% agarose gel electrophoresis, whilst the A allele was not digested by *MspI*. The 199 bp *TP53* Arg72Pro PCR products were amplified with the primers 5′-TTGCCGTCCCAAGCAATGGATGA-3′(sense) and 5′-TCTGGGAAGGGACAGAAGATGAC-3′(antisense) and digested with *BstUI*. The Arg allele revealed 113 and 86 bp fragments following digestion and 2% agarose gel electrophoresis, whilst the Pro allele was not digested by *BstUI*.

All of the PCR reactions were carried out by a Peltier Thermal Cycler (PTC 200), MJ Research, in a final volume of 20ul containing 100ng of each primer, 50ng genomic DNA, 3mM $MgCl_2$, 0,5ul dNTPs (2mM) and 1 U of HotFireTaqPolimerase (Odex, Italy) in the buffer provided by the manufacturer.

The genotype screening was performed simultaneously for cases and controls. The results were regularly confirmed by random re-genotyping of more than 10% of the samples for each polymorphism analysed.

3.3 Statistical analyses

Genotype distribution for each polymorphism was tested in controls for Hardy-Weinberg equilibrium and differences in expected and observed frequencies were tested for statistical significance by Pearson chi-square test. Differences in baseline sociodemographic characteristics between cases and controls were analyzed using chi-square test and Student's t-test. Multivariate logistic regressions were used to examine the association between each genotype and risk of selected health endpoints (all CRC, colon cancer, and rectal cancer separately). Odds ratio (OR) and 95% confidence interval (CI) were estimated with matching factors (age, gender) and potential confounders (smoking) included in the models. Combinations of genotypes, selected from SNPs in genes of the same DNA repair pathway, were constructed and investigated for their possible impact on CRC risk. The size of the cohort enabled investigations of binary combinations only. Furthermore, stratified analyses were conducted to evaluate effects of potentially modifying factors such as age (the age groups based on tertiles of age distribution) and smoking status (smokers vs. non-smokers) on the associations of interest. All tests were two-sided and performed on 5% level of statistical significance. Statistical calculations were performed using SPSS 13.0 for Windows (Chicago, Il, USA).

4. Results

4.1 Studied population

The main characteristics of the subjects involved in the study are reported in **Table 1**.

The age range of individuals from the cohort was between 26-86 years old with a fairly similar mean age at the diagnosis for both CRC cases and controls (57.4±12.8 and 58.5±10.5 years, respectively). The stratification for the cancer site revealed a prevalence of patients with colon cancer (n=335) in comparison with patients with rectal cancer (n=197). However, the age range and the mean age at the diagnosis were similar in both subgroups. Based on the information collected in the questionnaires concerning the lifestyle habits, the smoking habit was the only confounder analyzed in this study. Smokers were considered individuals currently smoking at the time of the diagnosis or individuals who had quit smoking in the last 5 years from the moment of the diagnosis. The distribution of smokers/non-smokers was not different between the cases and controls, with a majority of individuals belonging to the category non-smokers (71.7% and 73.2% resp.). Other lifestyle factors were not included into the present exploratory study since they were either based on a limited number of observations or their reliability needed to be proven Therefore this information was not suitable for statistical analyses at the moment of compiling the present work.

4.2 Polymorphisms in DNA repair genes

Allelic frequencies for DNA repair gene polymorphisms are presented in **Table 2**, and are in agreement with previous reports on the central European population (Vodicka et al, 2004, 2006).

None of the studied polymorphisms was independently associated with CRC risk in either codominant (considering together individuals bearing at least one variant allele) or dominant (separating individuals with heterozygous and homozygous variant genotypes) model of inheritance (**Table 2**). An association of borderline significance (p=0.06) was observed for *APE1* Asn148Glu polymorphism. Individuals homozygous for the variant allele of the polymorphism exhibited a moderately increased risk of CRC (OR: 1.39, 95% CI 0.98-1.96).

When analyzing a specific cancer site (**Table 3**), the variant allele homozygous genotype for the *APE1* Asn148Glu polymorphism was associated with an increased risk of colon cancer (OR: 1.50, 95% CI 1.01-2.22; p=0.05). On the contrary, individuals bearing homozygous variant allele genotype for the Glu185Gln polymorphism in the *NBS1* gene exhibited a moderately decreased risk of colon cancer (OR: 0.65, 95% CI 0.40-1.04; p=0.07). When similar analyses were performed on patients with rectal cancer, no independent association with either polymorphism emerged (**Table 3**).

Genotype combination interactions

Binary genotype combination interactions were tested for association with CRC risk for selected SNPs in genes involved in the same DNA repair pathway. We found a significantly increased risk of CRC in individuals carrying variant allele homozygous genotypes for both *APE1* Asn148Glu and *hOGG1* Ser326Cys polymorphisms (OR: 6.37, 95% CI 1.40-29.02; p=0.02, **Table 4**). The same genotype combination also showed an increased risk for colon cancer (OR: 7.14, 95% CI 1.49-34.38; p=0.01, **Table 5**). Analysis for rectal cancer suggests that individuals bearing *XPD* 751GlnGln and *XPG* 110HisHis genotypes in combination may exhibit increased risk for this particular cancer (OR: 8.14, 95% CI 0.87-86.36; p=0.06), but there were only three individuals in the cases and one person in controls. This is likely due to the fact that rectal cancer group accounted for 197 individuals.

Interaction of genotypes and smoking habit

The modifying effect of smoking habit on association between DNA repair polymorphisms and CRC risk was evaluated by applying multivariate analyses, obtained for strata of smokers and non-smokers. A significant interaction was observed between smoking habits and the *hOGG1* Ser326Cys polymorphism. Smokers with variant allele homozygous genotype for the polymorphism showed an increased risk of CRC (OR: 4.17, 95% CI 1.17-15.54; p=0.03).

The modifying effect of smoking in the association between selected combinations of genotypes and CRC risk was evaluated in analoguous way as above. Smokers carrying variant allele homozygous genotype for the *XPC* Lys939Gln and heterozygous genotype for the *XPG* Asn1104His showed an increased risk of CRC

(OR: 5.95, 95% CI 1.27-29.14; p=0.03). Smoking patients carrying the *XPC* variant allele homozygous genotype and *XPD* heterozygous genotype combination exhibited an increased risk for colon cancer risk as compared to corresponding smoking controls (OR: 8.22, 95% CI 1.50-45.30; p=0.01).

Interaction with age

Cases and controls were stratified into three age groups of fairly similar size (tertiles of age distribution: 26-54 years, 55-63 years, and 64-86 years) to assess an influence of this variable. The association of *APE1* Asn148Glu polymorphism with increased CRC risk became more pronounced after the stratification for age. Both heterozygous and homozygous genotypes exhibited significantly increased risk of CRC (OR: 1.79, 95% CI 1.04-3.07; p=0.04, OR: 2.57, 95% CI 1.30-5.06; p=0.007, respectively) in the age group of 64-86 years. This association was particularly evident for colon cancer (OR: 1.86, 95% CI 0.99-3.50; p=0.05 for heterozygous genotype and OR: 3.02, 95% CI 1.41-6.49; p=0.005 for homozygous genotype). An association of borderline significance was observed also for *hOGG1* Ser326Cys variant genotype in the oldest group and both CRC (OR: 7.17, 95% CI 0.86-59.7; p=0.07) and colon cancer risk (OR: 8.19, 95% CI 0.96-70.14; p=0.06). Association with rectal cancer risk was recorded for *XPG* Asn1104His variant allele in the group of oldest (64-86 years) individuals with heterozygous (OR: 1.94, 95% CI 0.95-3.97; p=0.07) and homozygous (OR: 9.52, 95% CI 1.59-57.02; p=0.01) genotype.

4.3 Polymorphisms in cell cycle control genes

Allelic frequencies for cell cycle gene polymorphisms are presented in **Table 6,** and are determined for the particular population for the first time. None of the studied polymorphisms was independently associated with CRC risk in either codominant (considering together individuals bearing at least one variant allele) or dominant (separating individuals with heterozygous and homozygous variant genotypes) model of inheritance.

The stratification for the specific cancer site did not reveal any association of the selected polymorphisms with risk of colon or rectal cancer (**Table 7**).

Genotype combination interactions

We tested genotype combination interactions for association with CRC risk for SNPs in the selected genes involved in cell cycle control pathway. None of the analysed combinations was associated with an altered CRC risk. However, after stratification for cancer site, we found a significant association with increased risk of colon cancer in individuals carrying at least two variant alleles for both *TP53* PIN3 and *TP53* Arg72Pro polymorphisms (OR: 2.61, 95% CI 1.78-3.84; p≤0.0001, **Table 8**).

Due to a tight link between DNA repair and *TP53* genes, we analysed a possible interaction between *APE1* Asn148Glu and *TP53* PIN3 and Arg72Pro polymorphisms, respectively. No significant interaction was found for these combinations.

Table 1. Selected characteristics of CRC cases and healthy controls

	Controls (*n*=532)	**All cases (*n*=532)**	**Colon cancer (*n*=335)**	**Rectal cancer (*n*=197)**
Gender				
Males	294	294	167	127
Females	238	238	168	70
Age at diagnosis (years)				
Mean±SD	57.4±12.8	58.5±10.5	58.5±10.9	58.4±9.7
Range	29-85	26-86	26-84	26-86
Smoking status				
Non-smokers	71.7% (358)	73.2% (372)	75.6% (242)	69.1% (130)
Smokers	28.3% (141)	26.8% (136)	24.4% (78)	30.9% (58)

Table 2 Distribution of DNA repair polymorphisms and risk of CRC

Genotypes	Controls (n=532))	Cases (n=532)	OR (95% CI)	*P* value
Base-excision repair				
***XRCC1* Arg194Trp**				
ArgArg	466	454	1.00	
ArgTrp	59	72	1.24 (0.86-1.80)	0.25
TrpTrp	5	6	1.17 (0.35-3.87)	0.80
ArgTrp+TrpTrp	64	78	1.24 (0.87-1.77)	0.24
***XRCC1* Arg399Gln**				
ArgArg	219	229	1.00	
ArgGln	240	233	0.93 (0.72-1.21)	0.60
GlnGln	73	68	0.88 (0.60-1.29)	0.52
ArgGln+GlnGln	313	301	0.92 (0.72-1.18)	0.51
***hOGG1* Ser326Cys**				
SerSer	331	336	1.00	
SerCys	181	168	0.91 (0.70-1.18)	0.47
CysCys	20	28	1.43 (0.79-2.59)	0.24
SerCys+ CysCys	201	196	0.96 (0.75-1.23)	0.74
***APE1* Asn148Glu**				
AsnAsn	157	140	1.00	
AsnGlu	267	261	1.10 (0.83-1.47)	0.50
GluGlu	106	130	1.39 (0.98-1.96)	0.06
AsnGlu+GluGlu	373	391	1.18 (0.91-1.55)	0.22
Nucleotide-excision repair				
***XPD* Lys751Gln**				
LysLys	174	189	1.00	
LysGln	264	258	0.89 (0.68-1.17)	0.41
GlnGln	94	85	0.82 (0.57-1.18)	0.28
LysGln+GlnGln	358	343	0.87 (0.68-1.13)	0.30
***XPG* Asn1104His**				
AsnAsn	356	334	1.00	
AsnHis	153	177	1.25 (0.96-1.63)	0.10
HisHis	23	21	0.99 (0.54-1.83)	0.98
AsnHis+HisHis	176	198	1.22 (0.94-1.57)	0.13
***XPC* Lys939Gln**				
LysLys	189	171	1.00	
LysGln	243	268	1.23 (0.94-1.61)	0.14
GlnGln	100	93	1.02 (0.72-1.45)	0.90
LysGln+GlnGln	343	361	1.17 (0.90-1.50)	0.23

Double-strand break repair				
***XRCC3* Thr241Met**				
ThrThr	219	203	1.00	
ThrMet	250	264	1.14 (0.88-1.48)	0.32
MetMet	63	65	1.11 (0.75-1.65)	0.61
ThrMet+MetMet	313	329	1.13 (0.89-1.45)	0.32
***NBS1* Glu185Gln**				
GluGlu	239	246	1.00	
GluGln	220	234	1.03 (0.80-1.33)	0.83
GlnGln	71	52	0.71 (0.48-1.06)	0.10
GluGln+GlnGln	291	286	0.95 (0.75-1.21)	0.68

[a] Unconditional logistic regression analysis adjusted for age and sex.

Table 3 Distribution of DNA repair polymorphisms and risk of CRC in specific sites

Genotypes	Controls (n=532)	Colon (n=335)	OR (95% CI)	*P* value	Rectum (n=197)	OR (95% CI)	*P* value
Base-excision repair							
***XRCC1* Arg194Trp**							
ArgArg	466	288	1.00		166	1.00	
ArgTrp	59	45	1.24 (0.82-1.88)	0.32	27	1.24 (0.76-2.03)	0.38
TrpTrp	5	2	0.61 (0.12-3.21)	0.56	4	1.92 (0.51-7.31)	0.34
ArgTrp+TrpTrp	64	47	1.19 (0.79-1.78)	0.41	31	1.30 (0.82-2.07)	0.27
***XRCC1* Arg399Gln**							
ArgArg	219	152	1.00		77	1.00	
ArgGln	240	146	0.87 (0.65-1.16)	0.33	89	1.09 (0.76-1.56)	0.63
GlnGln	73	37	0.71 (0.45-1.11)	0.14	31	1.29 (0.78-2.12)	0.32
ArgGln+GlnGln	313	183	0.83 (0.63-1.09)	0.19	120	1.14 (0.81-1.59)	0.46
***hOGG1* Ser326Cys**							
SerSer	331	225	1.00		111	1.00	
SerCys	181	90	0.73 (0.53-0.98)	**0.03**	78	1.29 (0.91-1.81)	0.15
CysCys	20	20	1.52 (0.80-2.91)	0.20	8	1.22 (0.52-2.86)	0.65
SerCys+ CysCys	201	110	0.80 (0.60-1.07)	0.14	86	1.28 (0.92-1.79)	0.15
***APE1* Asn148Glu**							
AsnAsn	157	82	1.00		58	1.00	
AsnGlu	267	171	1.22 (0.87-1,69)	0.25	91	0.96 (0.65-1.42)	0.85
GluGlu	106	82	1.50 (1.01-2.22)	**0.05**	48	1.24 (0.79-1.96)	0.35
AsnGlu+GluGlu	373	153	1.30 (0.95-1.77)	0.10	139	1.05 (0.73-1.50)	0.81

Nucleotide-excision repair							
***XPD* Lys751Gln**							
LysLys	174	118	1.00		71	1.00	
LysGln	264	162	0.90 (0.66-1.22)	0.48	96	0.88 (0.62-1.27)	0.51
GlnGln	94	55	0.87 (0.58-1.31)	0.51	30	0.76 (0.46-1.24)	0.27
LysGln+GlnGln	358	217	0.89 (0.67-1.89)	0.43	126	0.85 (0.60-1.20)	0.36
***XPG* Asn1104His**							
AsnAsn	356	213	1.00		121	1.00	
AsnHis	153	113	1.26 (0.93-1.70)	0.13	64	1.22 (0.85-1.75)	0.27
HisHis	23	9	0.69 (0.31-1.53)	0.36	12	1.50 (0.72-3.11)	0.28
AsnHis+HisHis	176	122	1.19 (0.89-1.59)	0.24	76	1.26 (0.89-1.77)	0.19
***XPC* Lys939Gln**							
LysLys	189	105	1.00		66	1.00	
LysGln	243	176	1.30 (0.96-1.78)	0.09	92	1.11 (0.76-1.60)	0.59
GlnGln	100	54	0.97 (0.64-1.46)	0.88	39	1.11 (0.70-1.77)	0.65
LysGln+GlnGln	343	230	1.21 (0.90-1.61)	0.21	131	1.11 (0.78-1.57)	0.56
Double-strand break repair							
***XRCC3* Thr241Met**							
ThrThr	219	133	1.00		70	1.00	
ThrMet	250	162	1.07 (0.80-1.44)	0.65	102	1.27 (0.89-1.82)	0.18
MetMet	63	40	1.06 (0.67-1.66)	0.81	25	1.22 (0.71-2.09)	0.47

ThrMet+MetMet	313	202	1.07 (0.81-1.41)	0.64	127	1.26 (0.90-1.78)	0.18
***NBS1* Glu185Gln**							
GluGlu	239	154	1.00		92	1.00	
GluGln	220	151	1.07 (0.80-1.43)	0.65	83	0.97 (0.68-1.38)	0.87
GlnGln	71	30	0.65 (0.40-1.04)	0.07	22	0.80 (0.47-1.38)	0.43
GluGln+GlnGln	291	181	0.97 (0.73-1.27)	0.81	105	0.93 (0.67-1.29)	0.67

[a] Unconditional logistic regression analysis adjusted for age and sex.

Table 4 Selected SNP-SNP interaction for CRC cancers

Cases/Controls OR (95% CI)		***APE1* Asn148Glu genotypes**		
		Asn/Asn	Asn/Glu	Glu/Glu
	Ser/Ser	97/92 1	164/169 0.93 (0.65-1.32)	75/69 1.03 (0.67-1.59)
***hOGG1* Ser326Cys genotypes**	Ser/Cys	40/59 0.64 (0.39-1.04)*	86/86 0.94 (0.62-1.43)	42/35 1.16 (0.68-1.97)
	Cys/Cys	3/6 0.50 (0.12-2.06)	12/12 0.99 (0.42-2.31)	13/2 6.37 (1.40-29.02)**

* p=0.07
**p=0.02

Table 5 Selected SNP-SNP interaction for colon cancers

Cases/Controls OR (95% CI)		***APE1* Asn148Glu genotypes**		
		Asn/Asn	Asn/Glu	Glu/Glu
	Ser/Ser	59/92 1	116/169 1.08 (0.72-1.62)	50/69 1.15 (0.70-1.87)
***hOGG1* Ser326Cys genotypes**	Ser/Cys	21/59 0.57 (0.31-1.03)*	46/86 0.81 (0.50-1.33)	23/35 1.66 (0.57-1.98)
	Cys/Cys	2/6 0.57 (0.11-2.94)	9/12 1.22 (0.48-3.08)	9/2 7.14 (1.49-34.38)**

* p=0.06
**p=0.01

Table 6 Distribution of Cell-cycle polymorphisms and risk of CRC

Genotypes	Controls (n=544)	Cases (n=544)	OR (95% CI)	*P* value
***TP53* PIN3**				
A1A1	393	380	1.00	
A1A2	143	1572	1.10 (0.84-1.44)	0.69
A2A2	8	12	1.55 (0.63-3.84)	0.48
A1A2+A2A2	151	164	1.12 (0.86-1.46)	0.92
***TP53* Arg72Pro**				
ArgArg	285	287	1.00	
ArgPro	212	203	0.95 (0.74-1.22)	0.48
ProPro	45	53	1.17 (0.76-1.80)	0.34
ArgPro+ProPro	257	256	0.98 (0.78-1.26)	0.38
***CCND1* G/A870**				
GG	138	138	1.00	
GA	285	263	0.92 (0.69-1.23)	0.58
AA	119	143	1.20 (0.86-1.69)	0.29
GA+ AA	404	406	1.01 (0.76-1.32)	1.00

Table 7 Distribution of cell cycle polymorphisms and risk of CRC in specific sites

Genotypes	Controls (n=544)	Colon (n=341)	OR (95% CI)	*P* value	Rectum (n=202)	OR (95% CI)	*P* value
***TP53* PIN3**							
A1A1	393	238	1.00		142	1.00	
A1A2	143	94	1.08 (0.79-1.47)	0.60	58	1.12 (0.78-1.61)	0.53
A2A2	8	9	1.86 (0.71-4.88)	0.20	3	1.04 (0.27-3.97)	-
A1A2+A2A2	151	103	1.13 (0.84-1.52)	0.43	61	1.12 (0.78-1.59)	0.54
***TP53* Arg72Pro**							
ArgArg	285	171	1.00		116	1.00	
ArgPro	212	137	1.08 (0.81-1.43)	0.61	66	0.76 (0.54-1.09)	0.13
ProPro	45	32	1.19 (0.73-1.94)	0.50	21	1.15 (0.65-2.01)	0.63
ArgPro+ProPro	257	169	1.10 (0.84-1.44)	0.51	87	0.83 (0.60-1.15)	0.27
***CCND1* G/A870**							
GG	138	85	1.00		53	1.00	
GA	285	166	0.73 (0.53-0.98)	0.74	97	0.89 (0.60-1.31)	0.54
AA	119	90	1.52 (0.80-2.91)	0.30	53	1.16 (0.74-1.82)	0.52
GA+ AA	404	256	0.80 (0.60-1.07)	0.86	150	0.97 (0.67-1.40)	0.86

Table 8 Selected SNP-SNP interaction for colon cancer

Cases/Controls OR (95% CI)		***TP53* PIN3 genotypes**	
		A1/A1	A1/A2+A2/A2
***TP53* Arg72Pro genotypes**	Arg/Arg	227/84	58/14
		1	0.65 (0.35-1.23)
	Arg/Pro+Pro/Pro	166/81	91/88
		1.31 (0.92-1.90)	2.61 (1.78-3.84)*

*p≤0.0001

5. Discussion

While hereditary CRC is associated with high-penetrance mutations in several genes, the genetic determinants of the disease at population levels remain to be understood. Polymorphisms in critical genes can potentially alter the susceptibility to different cancers including CRC. The role of genetic variants (e.g. SNPs) in genes encoding key players in the susceptibility to the sporadic CRC is not satisfactorily clarified at present. One of the key players in CRC risk seems to be *MTHFR* C677T (Kono and Chen, 2005), along with polymorphisms in genes involved in metabolism and inflammatory processes. Recent years have also evidenced growing attention devoted to the role of DNA repair genes as CRC risk modulators. Interindividual variations in DNA repair genes may confer altered DNA repair capacity, and thus an enhanced cancer risk (Berwick ans Vineis, 2005).

In this study, we tested the hypothesis, whether SNPs in the genes encoding different DNA repair enzymes influence the risk of CRC. A further interest was devoted in exploring the possible modulating role of cell cycle control gene polymorphisms, but the results presented in this work are still preliminary. The study population was drawn from the Czech Republic, a country with one of the highest incident rates for colorectal cancer and the highest incidence rate for rectal cancer (Boyle and Langman, 2000, Janout and Kollarova, 2001, Parkin et al, 2005).
The strength of the present study is that (a) the cases and controls matched for age and sex, confounding factors known to introduce substantial bias in association studies (Wacholder et al, 2004); (b) an adequate size of the cohorts; (c) a representative character of the cohorts for the entire country (and, basically, for central Europe), a very homogeneous area, with generally typical lifestyle and dietary habits, in particular over the last 50-60 years; (d) several genetic polymorphisms, involved in relevant DNA repair and cell cycle control pathways assayed for simultaneously; (e) the inclusion of only colonoscopically negative individuals as controls; even though this selection may not necessarily represent the general population, but it does ensure disease-free control individuals.
The data analysis showed that none of the DNA repair polymorphisms included in this study was associated with the risk modulation of CRC. However, homozygote

carriers of variant allele of the Asn148Glu polymorphism in the BER *APE1* gene were at a marginally increased risk of the disease. The stratification of cases according cancer site pointed effect of 148Glu homozygous genotype confined to colon cancer. Interestingly, an observed increased risk in individuals, homozygous for variant alleles of the *APE1* Asn148Glu and *hOGG1* Ser326Cys polymorphisms, in both CRC and colon cancer indicated multiplicative gene-gene interaction. This interaction between genes involved in BER is probably suggestive of a modulating role for inflammatory processes/oxidative stress in colon cancer (Sanders et al., 2004). The *APE1* and *hOGG1* genes are known to repair oxidative DNA damage as a part of BER pathway (Weiss et al, 2005) and the relationships between the polymorphisms of these genes and functional outcomes have recently been highlighted in healthy subjects. An increased number of variant alleles for both *hOGG1* Ser326Cys and *APE1* Asn148Glu was associated with a significantly decreased repair of DNA oxidative damage (Vodicka et al., 2006). Similar to our findings, the Ser326Cys polymorphism of *hOGG1* was the only one (out of 12 investigated polymorphisms in BER genes) that showed a significant association with an increased risk of CRC in a Spanish cohort of CRC patients (Moreno et al., 2006). Although *hOGG1* Ser326Cys is one of the most frequently analyzed BER polymorphisms, the outcomes remain inconclusive (Kim et al, 2003; Hansen et al, 2005). On the other hand, there is only one report on *APE1* polymorphism on the CRC risk, where no significant association was found (Moreno et al., 2006).

In general, none of the SNPs in any of the DNA repair genes have so far been strongly associated with adenoma or CRC risk (Bigler et al, 2005; Stern et al, 2005; Moreno et al., 2006). The outcomes of different studies vary substantially, as recently reviewed by Naccarati et al. (2007). Our present results are in accordance with the observations that none of the individually analyzed DNA repair polymorphisms have been unambiguously associated with CRC risk so far. The number of total investigated genes and related polymorphisms in literature is extensive (almost 30 DNA repair genes for more than 70 polymorphisms). However, only few of them have been analysed in two and more studies and, in the majority of cases, a comparison of results to highlight general trends is not yet feasible. In general, there are no strong associations between DNA repair SNPs and adenoma or CRC risk, observed consistently in more investigations. Many studies

did not reveal any significant association at all. The majority of studies focused on polymorphisms in genes of BER and NER pathways, *XRCC1* and *XPD* in particular. Considering the putative subtle effect of many DNA repair polymorphisms, the impact of individual SNPs on CRC risk is indeed expected to be low. The current assumption is that the onset of sporadic CRC may be triggered by multiple environmental/life style factors with possible interaction with genetic factors like polymorphisms (De la Chapelle, 2004).

Although there is a weak association between single DNA repair polymorphisms, when assayed for adenoma and CRC risk, more realistic information may be provided by analyzing polymorphisms in combinations. Single SNPs in low-penetrance genes are unlikely to affect the susceptibility to cancer, but an "adverse combination" of less favorable genetic variants can exert and amplify a negative effect. However, analysis of SNPs in combination reduces the number of observations and decreases the statistical power of the studies. Only a few studies addressed DNA repair gene-gene interactions so far: particular combinations of *XPD* K751Q, *XRCC1* Arg399Gln and *XRCC3* Thr241Met wildtype genotypes were associated with an increased CRC risk in a cohort of 727 CRC patients and 736 controls from Taiwan (Yeh et al, 2005a). Investigations of more SNPs in the same DNA repair gene (especially for *XPD, ERCC1*, *EXO1*, and *MGMT*) are shown in **Tables 1-5** of the **Introduction**. In this context, haplotype studies appear as more informative. A haplotype, a set of closely linked alleles (SNPs), is inherited as a unit, ultimately covering the variability within a gene (International HapMap, 2005). Modulating effect of haplotypes was investigated 3-times for *XRCC1* combined polymorphisms (Arg194Trp, Arg280His and Arg399Gln). Specific *XRCC1* haplotypes increased the risk of CRC in interaction with alcohol intake (Hong et al, 2005a), and adenoma risk in concomitance with fatty acid intake (Stern et al, 2005). The same haplotype was associated with a decreased CRC risk in young individuals only (Moreno et al, 2006).

A critical analysis of the available association studies reveals several limitations, which have been possibly avoided in the present study. The most important critical point is associated with the often too small size of cohorts of cases and controls, resulting in a low statistical power and false, by chance, positive or negative outcomes. Several studies have showed the bias introduced by analyses performed in small populations (Wacholder et al, 2004). In this context, the number of studies

exceeding 500 cases, like the present cohort from Czech Republic, is currently still limited, both for colorectal adenoma and CRC (Kim et al, 2004a; Yeh et al, 2005a,b, and 2006). An additional important aspect concerns the inclusion (often disproportional) of different ethnic groups into the cases and the controls, with a subsequent obscuring of the outcomes. Different results may be expected due to intrinsic differences in the genetic background among Caucasians, Asians, African-Americans and other ethnic groups. Only in the most recent DNA repair association studies were the statistical analyses for the stratification of ethnicity included (Stern et al, 2005; Jin et al, 2005; Berndt et al, 2006, 2007; Goodman et al 2006; Huang et al, 2006).

The proper recruitment of cases and controls represents another key factor, which characterizes different studies. Whereas in adenoma and CRC patients we can follow the use of standard criteria for the diagnosis (e.g. colonoscopy, histological examination), a very complicated situation appears in recruited control individuals. The use of population-based unscreened control group does not prevent inclusion of individuals with undetected polyps, with subsequent attenuation of the study findings. The studies including only colonoscopically negative individuals may not be representative of the general population. They rather comprise individuals with any clear indication for colonoscopy, such as putative positive family history or any gastrointestinal problems. On the other hand, the major advantage of this clinic-based approach is obtaining the control group free of polyps or CRC. Another approach is the recruitment of only cancer-free individuals as a control population (i.e. individuals declaring no history of cancer in the past for them and for family, and/or individuals tested for cancer). Since it employs directed questionnaires, the reliability of answers should be considered and, if possible, verified. Besides, various cancer tests (expensive and laborious) are not yet completely reliable. Optimally, the use of two independent control groups (one screened for colonoscopy and one constituted by healthy general population) would minimize biases.

Proper matching cases and controls for age and sex were also rarely recorded. Modulating effects of age and sex on CRC onset may only be investigated by comparing matched cohorts. This was the main reason why we have restricted our cohort only to perfectly matched sex and age cases and controls.

The investigation of gene-environment interactions implies the simultaneous study of both environmental exposure and relevant genetic polymorphisms. While the genotyping methods are quite accurate, a reliable determination of the environmental exposure is both laborious and expensive (i.e. measurement of external/internal dose of a chemical or its metabolites) or unreliable and non-quantitative (i.e. information based on questionnaires only). In this present study, we attempted to address a major environmental/life style factor, smoking, and one major confounder, age. However, an inclusion of other variables (planned as a prospect) would have decreased the statistical power, after stratification of the cohort. We found a significant interaction between smoking habit and the *hOGG1* Ser326Cys polymorphism. The smokers carrying 326Cys/Cys genotype showed an increased risk of CRC as compared to control smokers. In another association study, based on 772 high-risk adenomas cases and 777 controls, a particular combination of three linked nonsynonymous polymorphisms in *XPC* (Arg492His, Ala499Val, and Lys939Gln) increased smoking related risk for colorectal adenoma (Huang et al, 2006). Similarly, in this present study, smoking patients carrying *XPC* 939GlnGln and *XPD* 751LysGln combined genotypes exhibited an increased risk for colon cancer risk.

Age also seems to be a relevant factor affecting the association between *APE1* Asn148Glu polymorphism and an increased CRC risk, as the effect of above polymorphism was most pronounced in individuals between 64 and 86 years old. The *XPG* Asn1104His polymorphism exhibited an association with rectal cancer risk in the group of oldest individuals included in the study. This phenomenon is difficult to explain, however, as many influencing factors can cause an increased susceptibility during a lifetime. An unsubstantiated explanation could be differential accumulation of DNA damage due to different genotypes. This is likely to increase rather among older individuals as well as consequences of time, or as an exhaustion of compensatory repair efficiency in the case of adverse genotype in one or two repair genes with functional impact. Similarly, Jin et al (2005), found that *XRCC3* 241Met allele was associated with CRC risk among older individuals (>60 years). On the other hand, several studies reported an increased susceptibility to adenoma or CRC risk in younger individuals (Bigler et al, 2005; Yeh et al, 2005a; Moreno et al, 2006). Larger and well-designed studies are needed to address the role of DNA repair polymorphisms and ageing in CRC risk.

Due to the close link with DNA repair, we have also tested the hypothesis whether SNPs in genes encoding some essential cell cycle control enzymes may influence the risk of CRC. The data analysis showed that none of the polymorphisms included in the present study was independently associated with the risk modulation of CRC, even after stratification for cancer site. However, when polymorphisms were analysed in binary combinations, individuals carrying at least two variant alleles for both *TP53* PIN3 and *TP53* Arg72Pro exhibited significantly increased risk of colon cancer. These results are in agreement with observations from previous studies: none of the analyzed cell cycle polymorphism has been unambiguously associated with CRC risk so far (Bai and Zhu, 2006; Knudsen et al, 2006). Several studies associated *TP53* and *CCND1* polymorphisms to different kind of cancers (including CRC). However, in the case of *CCND1*, associations with cancer susceptibility have been observed for both wildtype and variant alleles in different studies. The majority of studies link the *CCND1* 870A allele to increased cancer risk and poor disease outcome. On the other hand, other studies have associated the 870G allele with an increased cancer risk, and others have not reported any significant association with any allele of the G/A870 polymorphism (Grieu et al., 2003; Le Marchand et al., 2003; Hong et al., 2005b; Jiang et al., 2006; Probs-Hensch et al., 2006; Schernhammer et al., 2006). A similar controversial situation can be described for *TP53* gene polymorphisms. Functionally significant changes have been reported for the different genotypes in the most frequently investigated polymorphism of *TP53* gene (Arg72Pro). In some studies, the variant allele has been clearly associated with the age of onset of cancer and its prognosis. However, whether these variants are associated with an altered cancer risk is not currently clear at present (Olschwang et al, 1991; Kawajiri et al, 1993, Sjalander et al, 1995;Gemignani et al, 2004; Koushik et al, 2006). Due to multiple variants in this pathway, modulating effect of these SNPs would require additional, complex analyses with simultaneous addressing functional aspects. As a first step, analyses of haplotypes would represent much more powerful approach than single polymorphism investigations.

This present PhD work is a part of a wider project in collaboration with other Czech and European Research Institute. The aim of the project is to assess the possible associations of numerous SNPs of genes in several different regulatory pathways with CRC risk. In particular, the screening of polymorphisms of genes involved in

metabolism of the xenobiotics, of polymorphisms in bilirubin genes or insulin-like growth factors are at present almost completed. The size of initial cohort of cases and controls from the Czech Republic is also currently increasing, since the sampling is still ongoing. The possibility to reach a cohort of more than 1000 cases and 1000 controls is desirable, in order to perform more complex investigations, with an adequate statistical power, even when the frequency of alleles is relatively low. Future studies have also to take into consideration the role of haplotypes, which may correlate with a disease, while single SNPs may not.

The complex etiology of CRC and observed high incidence in Czech Republic stresses an importance of a systematic approach by combining epidemiological and molecular biological methods on large cohorts, to understand critical pathways in colorectal tumorigenesis. Yet, several other factors, contributing to CRC, such as microbial flora, inflammatory processes, stool composition etc., have to be simultaneously addressed.

6. Conclusions

The major outcomes of the present PhD thesis may be briefly summarized as follows:

- None of the DNA repair and cell cycle genetic polymorphisms included in this study was strongly associated with the risk modulation of CRC. Individuals with homozygous variant genotype in *APE1* Asn148Glu polymorphism (BER pathway) were at a marginally increased risk of sporadic CRC. Above association was particularly pronounced in patients with colon cancer.
- Individuals simultaneously homozygous for variant alleles of the *APE1* Asn148Glu and *hOGG1* Ser326Cys polymorphisms were at significantly increased risk of CRC. This association was particularly pronounced in colon cancer patients and is indicative of multiplicative gene-gene interactions. The interaction between genes involved in BER is probably suggestive of a modulating role for inflammatory processes/oxidative stress in colon cancer. A significant association was also found with increased risk of colon cancer, in individuals carrying at least two variant alleles for both *TP53* PIN3 and *TP53* Arg72Pro polymorphism.
- A significant interaction was revealed between smoking habit and *hOGG1* Ser326Cys polymorphism. The smokers carrying 326Cys/Cys genotype showed an increased risk of CRC. Age also seems to be a relevant factor affecting the association between *APE1* Asn148Glu and an increased CRC risk, as the effect of above polymorphism was most pronounced in older individuals. *XPG* Asn1104His polymorphism exhibited an association with rectal cancer risk in the group of oldest individuals included in the study.

CRC is a multifactorial disease which is the result of complex interactions between the individual genetic background and the external/internal environmental factors during the lifetime. The present results show a possible subtle effect of particular polymorphisms of DNA repair and cell cycle genes in modulating the CRC risk, which may be visible and supported by sufficient statistical power only on large cohorts of individuals. A role of SNPs in modifying the individual susceptibility to cancer may be more pronounced in specific tumor sites, and in concomitance with other polymorphisms and lifestyle factors, as well as age.

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9. List of publications and abstracts

Publications

- Vodicka P, Stetina R, Polakova V, Tulupova E, Naccarati A, Vodickova L, Kumar R, Hanova M, **Pardini B**, Slyskova J, Musak L, De Palma G, Soucek P, Hemminki K. Association of DNA repair polymorphisms with DNA repair functional outcomes in healthy human subjects. Carcinogenesis *2*006, Epub ahead of print.

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- M. Ballardin, C. Cerri, M. Cipollini, L. Migliore, A. Naccarati, **B. Pardini**, A.M. Rossi, R. Barale (2003) *Danno al DNA indotto dalla radio-iodio terapia e possibile effetto protettivo di antiossidanti naturali.* Atti del 5° Convegno FISV, Rimini (RN), Oct 10-13 2003.
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- P. Vodicka, M. Hanova, E. Tulupova, V. Polakova, A. Naccarati, **B. Pardini**, L. Vodickova, P. Soucek, J. Novotny, K. Hemminki; Molecular *Epidemiology as tool for understanding sporadic colorectal cancer*. Abstract from Annual Meeting of the EEMS, Prague, Czech Republic, July 2-6 2006
- V. Polakova, M. Hanova, E. Tulupova, J. Slyskova, A. Naccarati, **B. Pardini**, P. Vodicka, L. Vodickova, J. Novotny, K. Hemminki; *Molecular and genetic characteristics of sporadic colorectal cancer in the Czech Republic.* Abstract from Annual Meeting of the EEMS, Prague, Czech Republic, July 2-6 2006
- **B. Pardini**, R. Barale, P. Vodicka, E. Tulupova, A. Naccarati, L. Vodickova, J. Novotny; *Genetic polymorphism in the MDR-1 gene encoding for P-glycoprotein drug transporter and susceptibility to colorectal cancer*. Abstract from Annual Meeting of the EEMS, Prague, Czech Republic, July 2-6 2006
- V. Polakova, R. Stetina, M. Hanova, E. Tulupova, A. Naccarati, L. Vodickova, **B. Pardini**, J. Slyskova, L. Musak, K. Hemminki, P. Vodicka; *Relationship between DNA repair polymorphisms in XRCC1 gene and the repair rates induced by Gamma-irradiation* Abstract from DNA REPAIR 2006, Hamburg, Germany; September 12-15 2006
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